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# Canadian Journal of Fisheries and Aquatic Sciences

# Journal canadien des sciences halieutiques et aquatiques

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# A General Framework for Developing Sequential Fisheries Models

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Schnute, J.T. 1994. A general framework for developing sequential fisheries models. Can. J. Fish. Aquat. Sci. 51: 1676–1688.

A sequential fisheries model relates observed data to the biological dynamics of an underlying stock. Either model component, dynamic or observational, can be subject to statistical variation. Current fisheries literature includes models with (1) variable dynamics and no observation error, (2) deterministic dynamics and observations subject to measurement error, and (3) combined dynamic and measurement variability. This paper presents a general framework for developing sequential fishery models and estimating model parameters from available data. The framework encompasses most traditional stock assessment models and suggests new, potentially useful extensions. It generalizes the conventional definition of state space model to include nonlinear equations with nonnormal error. The paper rigorously compares two paradigms (KF: Kalman filter, EV: errors in variables) used for parameter estimation. Each paradigm is formulated in both frequentist and Bayes contexts, where Bayes is shown to be most appropriate for the EV paradigm. Model design concepts are illustrated with a simple example oriented to catch data and a more complex example with catch-at-age data. Because the framework forces essential questions to be asked about underlying processes, observed data, and sources of variability, it can help delineate the limits of current knowledge and establish rational priorities for future data collection.

Un modèle séquentiel de pêche met en relation les données observées de la dynamique biologique d'un stock donné. L'un ou l'autre des éléments du modèle, soit la dynamique et les données observées, peut être sujet à une variation statistique. Dans la littérature actuelle, on retrouve des modèles avec 1) une dynamique variable et aucune erreur d'observation, 2) une dynamique déterministe et des observations sujettes à des erreurs de mesure et 3) une dynamique combinée et la variabilité des mesures. Dans le présent article, on présente un schéma général pour élaborer des modèles de pêche séquentiels et pour évaluer les paramètres du modèle à l'aide des données disponibles. Ce schéma s'applique à la plupart des modèles traditionnels d'évaluation des stocks et peut avoir d'autres applications utiles. On généralise la définition habituelle d'un modèle d'espace d'états de façon à inclure des équations non linéaires et des erreurs non normales. Dans cet article, on compare rigoureusement deux paradigmes (KF : filtre de Kalman et EV : erreurs dans les variables) utilisés pour évaluer les paramètres. Chaque paradigme est formulé en termes de fréquences et selon la méthode de Bayes; cette dernière méthode semble être la plus pertinente dans le cas du paradigme EV. Les concepts liés à l'élaboration du modèle sont illustrés à l'aide d'un exemple simple faisant appel à des données concernant les prises et à l'aide d'un exemple plus complexe faisant appel à des données concernant les prises selon l'âge. Comme ce schéma nous force à poser des questions essentielles sur les processus en jeu, les données observées et les sources de variabilité, il peut nous aider à fixer les limites de nos connaissances actuelles et à établir des priorités rationnelles concernant la collecte future des données.

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Most fishery models include a component that relates current and past population sizes. For example, stock–recruitment models sequentially express recruitment as a function of previous stock sizes. Similarly, catch-at-age models relate the number of fish at age $a$ in year $t$ to the number at age $a - 1$ in year $t - 1$. This paper presents a general framework for developing sequential fishery models and estimating model parameters from available data. The framework encompasses most traditional stock assessment models and suggests new, potentially useful extensions.

Mathematically, the analysis here draws heavily on state space models, as described by Gelb (1974), Aoki (1987), and Harvey (1989). However, because traditional assumptions about normal error are too restrictive for many fishery models, the framework here is somewhat broader than usual. Readers can treat this paper as a road map to the subject, where map boundaries are deliberately chosen large enough to put the geography into perspective.

Fisheries literature includes three categories of models that can be distinguished by statistical assumptions regarding biological dynamics and observation. In the first category, model parameters completely determine the stock dynamics, and statistical variation is presumed due only to measurement errors in the observed data. For example, an analysis of catch-at-age data may begin with parameters that describe recruitments, mortalities, and catchabilities. These parameters then determine the population age structure for all years $t = 1,\dots,T$, via sequential equations of population dynamics. The resulting theoretical age structure can then be compared with the observed age structure, and the parameters can be adjusted to obtain an optimal fit. Both the multinomial (Fournier and Archibald 1982; Methot 1990) and

Table 1. Models in this paper, rated by two criteria: linear or nonlinear; normal or not normal. Deterministic models are considered special cases of the normal, in which all variances are zero (0). Some models are stated generically; others provide examples related to fishery data. Thus, models F1–F4 are special cases of G1–G4, respectively. Model F1 is a variant of F2 in which (5.13) replaces (CA.11), (CA.14), and (CA.16).

| Linear? | Normal? | Equations | Label | Description |
|---|---|---|---|---|
| No | No | (2.5a)–(2.5c) | G1 | Generic |
| No | Yes | (1–3)–(1.6) or (2.1)–(2.4) | G2 | Generic |
| No | 0 | (1.1)–(1.2) | G3 | Generic |
| Yes | Yes | (1.7)–(1.9) | G4 | Generic |
| No | No | See table caption | F1 | Fishery: catch, age, index |
| No | Yes | (CA.1)–(CA.16) | F2 | Fishery: catch, age, index |
| No | 0 | (CA.1)–(CA.11) | F3 | Fishery: catch, age, index |
| Yes | Yes | (1.10)–(1.12) | F4 | Fishery: catch, index |

lognormal (Deriso et al. 1985) likelihood functions have been used as the basis for such a comparison. Megrey (1989) reviews much of the literature on catch-at-age analysis.

The second category of models assumes that the sequential population equations include process error and that the observations are exact. For example, Schnute (1989) examines several variants of Schaefer's (1957) model from this point of view, and demonstrates that the results depend significantly on how the process error is introduced. More generally, various delay-difference models have been proposed (Deriso 1980; Schnute 1985, 1987) that readily accommodate process error. Hilborn and Walters (1992, chap. 9) review this literature; they also discuss variants of these models with measurement error only, as in the first category above.

The third category combines the previous two by incorporating both measurement and process errors. Ludwig and Walters (1981) illustrate this approach in the context of the Ricker's (1954) description of stock–recruitment data. They show that parameter estimates depend crucially on the presumed ratio of measurement to process error. Similarly, Schnute et al. (1989) and Schnute (1991, 1993) illustrate the importance of this error ratio in models of catch and catch per unit effort (CPUE) data. Mendelssohn (1988), Sullivan (1992), and Gudmundsson (1994) carry this analysis a significant step further by demonstrating how to incorporate both measurement and process error into catch-at-age and catch-at-length models.

Ludwig and Walters (1981) and Sullivan (1992) illustrate two distinct paradigms used to analyse models that include both types of error. The former paper finds its roots in the functional relationship defined by Kendall and Stuart (1979, sect. 29.14). From this point of view, population sizes are unknown parameters to be estimated along with other model parameters. By contrast, Sullivan (1992) treats population sizes as unknown random variables whose distributions are determined by other model parameters, as in a structural relationship (Kendall and Stuart 1979, sect. 29.6). I refer to the approach by Ludwig and Walters (1981) as an "errors in variables" (EV) paradigm, where the name derives from the classical estimation problem for a functional relationship. Similarly, I refer to Sullivan's (1992) approach as a "Kalman filter" (KF) paradigm because the recursive estimates of the Kalman filter are used to build the model's likelihood function.

The framework presented in this paper includes a complete, rigorous description of both the KF and EV approaches to model development. It encompasses all the literature discussed above, and suggests new, potentially useful extensions. Models within this framework can be linear or nonlinear. Specialized results are presented for models with normal error, although the EV approach can accommodate almost any reasonable error assumptions. Section 1 defines state space models, where the definition here is somewhat broader than usual. Section 2 presents a general method for constructing both likelihood functions and Bayes posteriors for state space models. The KF and EV paradigms are described in Sections 3 and 4, respectively, where it is shown that the EV paradigm can be most rigorously defined in a Bayes framework. A simple fishery model is discussed throughout the paper, but this is extended to a more complex catch-at-age model in Section 5. Section 6 closes with a discussion of the discipline and flexibility that the framework imposes on fishery model design. Because essential questions must be addressed about underlying processes, observed data, and sources of variability, state space models can help delineate the limits of current knowledge and establish rational priorities for future data collection.

A general model framework necessarily requires an appropriate level of abstraction, which is achieved here through the vector and matrix notation presented in Sections 1 and 2. Readers interested primarily in fishery model design may wish to skip Sections 3 and 4 on first reading. By contrast, readers concerned with parameter estimation may focus primarily on the theory developed in Sections 3 and 4. Table 1, which summarizes all models in this paper, serves as a map of the mathematical topography. I recommend that readers consult this map frequently to keep the discussion in perspective.

## 1. State Space Models

A state space model typically involves three time-dependent vectors: a state vector $\mathbf{X}_t$ of dimension $m$, an observation vector $\mathbf{Y}_t$ of dimension $n$, and a control vector $\mathbf{Z}_t$ of dimension $r$. Also, the model includes a time-independent parameter vector $\mathbf{\Theta}$ of dimension $k$. The states $\mathbf{X}_t$ represent various unobserved population characteristics at time $t$, such as total biomass or numbers of fish at specified ages. The observations $\mathbf{Y}_t$ correspond to quantities actually observed at time $t$, such as catch per unit effort or proportions of fish at specified ages. The controls $\mathbf{Z}_t$ also comprise data observed at time $t$; however, unlike the observations $\mathbf{Y}_t$, the controls do not depend directly on the states $\mathbf{X}_t$. For example, $\mathbf{Z}_t$ might include the catch at time $t$, which acts as a known control on

TABLE 2. Vector and matrix quantities in the linear (L) and nonlinear (N) normal models (1.3)–(1.6) and (1.7)–(1.9), respectively.

| Quantity | Dimensions | Model | Meaning |
|---|---|---|---|
| $\mathbf{A}_t$ | $m \times 1$ | L | Process offset |
| $\mathbf{B}_t$ | $m \times m$ | L | Process transition matrix (state to state) |
| $\mathbf{C}_t$ | $n \times 1$ | L | Observation offset |
| $\mathbf{D}_t$ | $n \times m$ | L | Transformation matrix (state to observation) |
| $\mathbf{F}$ | $m \to m$ | N | Process transition function (state to state) |
| $\mathbf{G}$ | $m \to n$ | N | Transformation function (state to observation) |
| $\mathbf{U}_t$ | $m \times m$ | L, N | Process covariance matrix |
| $\mathbf{V}_t$ | $n \times n$ | L, N | Observation covariance matrix |
| $\mathbf{X}_t$ | $m \times 1$ | L, N | State vector |
| $\mathbf{Y}_t$ | $n \times 1$ | L, N | Observation vector |
| $\mathbf{Z}_t$ | $r \times 1$ | L, N | Control vector |
| $\boldsymbol{\delta}_t$ | $m \times 1$ | L, N | Process error |
| $\boldsymbol{\epsilon}_t$ | $n \times 1$ | L, N | Observation error |
| $\boldsymbol{\Theta}$ | $k \times 1$ | L, N | Parameter vector |

the population dynamics. Other components of $\mathbf{Z}_t$ might be environmental observations that influence either the population dynamics or the process of observing $\mathbf{Y}_t$.

In deterministic form, a state space model consists of two vector equations involving $\mathbf{X}_t$, $\mathbf{Y}_t$, $\mathbf{Z}_t$, and $\boldsymbol{\Theta}$. The process equation, also called the dynamic state equation,

$$(1.1) \qquad \mathbf{X}_t = \mathbf{F}(\mathbf{X}_{t-1},\mathbf{Z}_t,\boldsymbol{\Theta})$$

relates the state vector at time $t$ to the previous state vector at time $t-1$. The observation equation

$$(1.2) \qquad \mathbf{Y}_t = \mathbf{G}(\mathbf{X}_t,\mathbf{Z}_t,\boldsymbol{\Theta})$$

relates the observations to the states at time $t$. The function $\mathbf{F}$ in (1.1) corresponds to a set of $m$ equations, one for each component of the state vector $\mathbf{X}_t$. Similarly, $\mathbf{G}$ in (1.2) is given by a set of $n$ equations. Both $\mathbf{F}$ and $\mathbf{G}$ can be nonlinear functions of their arguments. The model can be rendered explicitly time dependent by including time $t$ as one component of the control vector $\mathbf{Z}_t$.

Although model (1.1)–(1.2) incorporates only one time lag in the dynamic equation (1.1), this is not a serious restriction. The state vector itself can be designed to include lags of population variables. For example, the state vector for a salmon population with a 2-yr life cycle might be

$$\mathbf{X}_t = \begin{pmatrix} P_t \\ P_{t-1} \end{pmatrix},$$

where $P_t$ denotes population size in year $t$. A 1-yr lag in this state vector includes a 2-yr lag in $P_t$.

Practical application of the deterministic model (1.1)–(1.2) requires additional assumptions about statistical variation. For example, the "normal" model

$$(1.3) \qquad \mathbf{X}_t = \mathbf{F}(\mathbf{X}_{t-1},\mathbf{Z}_t,\boldsymbol{\Theta}) + \boldsymbol{\delta}_t$$

$$(1.4) \qquad \mathbf{Y}_t = \mathbf{G}(\mathbf{X}_t,\mathbf{Z}_t,\boldsymbol{\Theta}) + \boldsymbol{\epsilon}_t$$

adds multivariate normal error to both the process and observation equations. Thus, the $m$-dimensional random vector $\boldsymbol{\delta}_t$ in (1.3) is presumed multinormal with mean 0 and covariance matrix

$$(1.5) \qquad \mathbf{U}_t = \mathbf{U}(\mathbf{Z}_t,\boldsymbol{\Theta})$$

that may depend on the control and parameter vectors. Similarly, the $n$-dimensional random vector $\boldsymbol{\epsilon}_t$ in (1.4) is presumed multinormal with mean 0 and covariance matrix

$$(1.6) \qquad \mathbf{V}_t = \mathbf{V}(\mathbf{Z}_t,\boldsymbol{\Theta}).$$

The functions $\mathbf{U}$ and $\mathbf{V}$ in (1.5)–(1.6) are matrix functions of their arguments; thus, $\mathbf{U}$ and $\mathbf{V}$ determine symmetric matrices with dimensions $m \times m$ and $n \times n$, respectively. The random vectors $\boldsymbol{\delta}_t$ and $\boldsymbol{\epsilon}_t$ in (1.3)–(1.4) are assumed both independent of each other and independent for different times $t$.

In summary, the normal model is completely specified by the vectors $\mathbf{X}_t$, $\mathbf{Y}_t$, $\mathbf{Z}_t$, and $\boldsymbol{\Theta}$, and the four vector functions $\mathbf{F}$, $\mathbf{G}$, $\mathbf{U}$, and $\mathbf{V}$ in (1.3)–(1.6), respectively. At each time $t$, the model describes an underlying population with $m$ state variables that lead to $n$ items of observed data. An additional time series $\mathbf{Z}_t$ of $r$ observations may influence both the population dynamics (1.3) and the observation process (1.4). Conceptually, the model reflects the practical reality of most fisheries, where an unseen population gives rise to data that can actually be collected. The parameter vector $\boldsymbol{\Theta}$ typically includes (1) biological and fishery parameters, such as mortalities and catchabilities, (2) variances needed to specify $\mathbf{U}$ and $\mathbf{V}$ in (1.5)–(1.6), and (3) initial states needed to start the dynamic process (1.3).

The "linear normal" model is an important special case of (1.3)–(1.6) obtained by assuming that $\mathbf{F}$ and $\mathbf{G}$ are linear functions of the state vector $\mathbf{X}$. In this case, (1.3)–(1.4) can be written as

$$(1.7) \qquad \mathbf{X}_t = \mathbf{A}_t + \mathbf{B}_t\mathbf{X}_{t-1} + \boldsymbol{\delta}_t$$

and

$$(1.8) \qquad \mathbf{Y}_t = \mathbf{C}_t + \mathbf{D}_t\mathbf{X}_t + \boldsymbol{\epsilon}_t,$$

respectively, where $\mathbf{A}_t$, $\mathbf{B}_t$, $\mathbf{C}_t$, and $\mathbf{D}_t$ denote matrices defined in terms of $\mathbf{Z}_t$ and $\boldsymbol{\Theta}$:

$$(1.9) \qquad \begin{gathered} \mathbf{A}_t = \mathbf{A}(\mathbf{Z}_t,\boldsymbol{\Theta}),\ \mathbf{B}_t = \mathbf{B}(\mathbf{Z}_t,\boldsymbol{\Theta}) \\ \mathbf{C}_t = \mathbf{C}(\mathbf{Z}_t,\boldsymbol{\Theta}),\ \mathbf{D}_t = \mathbf{D}(\mathbf{Z}_t,\boldsymbol{\Theta}). \end{gathered}$$

Table 2 summarizes the vector and matrix objects defined in the normal models (1.3)–(1.6) and (1.7)–(1.9).

To illustrate the linear normal model, consider a fishery from which the only available data are catch biomass $C_t$ and a biomass index $I_t$ (such as catch per unit effort) for each year $t$. Within the underlying fish population, let $R_t$ and $S_t$ denote biomasses at the start of year $t$ corresponding, respectively, to new recruits and survivors from previous generations. Perhaps the simplest possible model for this situation is defined by the three equations

$$(1.10)\quad R_t = R_{t-1} + \delta_{1t}$$

$$(1.11)\quad S_t = s(R_{t-1} + S_{t-1} - C_{t-1}) + \delta_{2t}$$

$$(1.12)\quad I_t = q\left(R_t + S_t - \frac{1}{2}C_t\right) + \epsilon_{1t},$$

where $\delta_{1t}$, $\delta_{2t}$, and $\epsilon_{1t}$ are independent normal variates with mean 0 and variances $\sigma_1^2$, $\sigma_2^2$, and $\tau^2$, respectively.

The survival equation (1.11) relates surviving biomass $S_t$ at the start of year $t$ to total biomass minus catch in year $t - 1$. The parameter $s$ is a biomass coefficient of natural survival. For example, $s$ might be constant in a population for which fish that grow rapidly also experience higher natural mortality. The rather unconventional recruitment equation (1.10) assumes that recruitment each year deviates randomly from the previous recruitment. This allows the population to drift to high or low values over long periods, consistent with the observed dynamics of some fish populations. When $\sigma_1 = 0$, the model reduces to constant recruitment. Finally, the observation equation (1.12) states that, with proportionality constant $q$, the observed index $I_t$ references the mean biomass during the fishery. Biomasses before and after fishing are assumed to be $R_t + S_t$ and $R_t + S_t - C_t$, whose mean is the expression in parentheses on the right-hand side of (1.12).

Model (1.10)–(1.12) is a linear normal state space model with state, observation, and control vectors.

$$(1.13)\quad \mathbf{X}_t = \begin{pmatrix} R_t \\ S_t \end{pmatrix}, \mathbf{Y}_t = (I_t), \mathbf{Z}_t = \begin{pmatrix} C_t \\ C_{t-1} \end{pmatrix}$$

as well as residual vectors

$$\boldsymbol{\delta}_t = \begin{pmatrix} \delta_{1t} \\ \delta_{2t} \end{pmatrix}, \boldsymbol{\epsilon}_t = (\epsilon_{1t}).$$

The system equations (1.7)–(1.8) are defined by the matrices

$$(1.14)\quad \begin{gathered} \mathbf{A}_t = \begin{pmatrix} 0 \\ -sC_{t-1} \end{pmatrix}, \mathbf{B}_t = \begin{pmatrix} 1 & 0 \\ s & s \end{pmatrix} \\ \mathbf{C}_t = \begin{pmatrix} -\frac{1}{2}qC_t \end{pmatrix}, \mathbf{D}_t = \begin{pmatrix} q & q \end{pmatrix} \end{gathered}$$

and the covariance matrices (1.5)–(1.6) are given by

$$(1.15)\quad \mathbf{U}_t = \begin{pmatrix} \sigma_1^2 & 0 \\ 0 & \sigma_2^2 \end{pmatrix}, \mathbf{V}_t = (\tau^2).$$

The diagonal matrix $\mathbf{U}_t$ implies that process errors for recruitment and survival are independent; more generally, $\mathbf{U}_t$ could be a symmetric nondiagonal matrix.

The model's complete parameter vector

$$(1.16)\quad \boldsymbol{\Theta} = (s, q, \sigma_1, \sigma_2, \tau, R_1, S_1)$$

can be divided into three groups: (1) deterministic parameters $(s,q)$, (2) standard errors $(\sigma_1, \sigma_2, \tau)$ of recruitment, survival, and observation, and (3) initial states $(R_1, S_1)$. Incidentally, the scalar catch $C_t$ in (1.13)–(1.15) should not be confused with the matrix $\mathbf{C}_t$ in (1.7)–(1.9). Throughout this paper, I consistently retain the generic matrix notation of Table 2, but otherwise I tailor notation to each particular example.

The model (1.10)–(1.12) assumes that catch biomass is known exactly and that, with different time lags, it influences both the population dynamics (1.11) and the observed index (1.12). Thus, the control vector $\mathbf{Z}_t$ in (1.13) includes both $C_t$ and $C_{t-1}$. Another feature of (1.10)–(1.12) is that it is not stationary. Although the conditional variate $R_t|R_{t-1}$ has a finite distribution, $R_t$ itself has infinite variance. This illustrates one of the advantages of state space models. They easily accommodate nonstationary behaviour, provided only that conditional distributions exist at each time step.

## 2. Model Likelihood

The normal model represents just one possible statistical counterpart to the deterministic state space model (1.1)–(1.2). More generally, the process equation (1.1) can be extended in any manner that defines the probability distribution $P(\mathbf{X}_t|\mathbf{X}_{t-1},\mathbf{Z}_t,\boldsymbol{\Theta})$ of the state vector $\mathbf{X}_t$ conditional on the previous state vector $\mathbf{X}_{t-1}$, as well as the control and parameter vectors. Similarly, the observation equation (1.2) can be extended statistically by defining $P(\mathbf{Y}_t|\mathbf{X}_t,\mathbf{Z}_t,\boldsymbol{\Theta})$. In the normal model, for example, the distributions

$$(2.1)\quad P(\mathbf{X}_t|\mathbf{X}_{t-1},\mathbf{Z}_t,\boldsymbol{\Theta}) = (2\pi)^{-\frac{m}{2}}|\mathbf{U}_t|^{-\frac{1}{2}} \exp\left[-\frac{1}{2}\boldsymbol{\delta}_t'\mathbf{U}_t^{-1}\boldsymbol{\delta}_t\right]$$

$$(2.2)\quad P(\mathbf{Y}_t|\mathbf{X}_t,\mathbf{Z}_t,\boldsymbol{\Theta}) = (2\pi)^{-\frac{n}{2}}|\mathbf{V}_t|^{-\frac{1}{2}} \exp\left[-\frac{1}{2}\boldsymbol{\epsilon}_t'\mathbf{V}_t^{-1}\boldsymbol{\epsilon}_t\right]$$

can be expressed in terms of model residuals

$$(2.3)\quad \boldsymbol{\delta}_t(\mathbf{X}_t,\mathbf{X}_{t-1},\mathbf{Z}_t,\boldsymbol{\Theta}) = \mathbf{X}_t - \mathbf{F}(\mathbf{X}_{t-1},\mathbf{Z}_t,\boldsymbol{\Theta})$$

$$(2.4)\quad \boldsymbol{\epsilon}_t(\mathbf{X}_t,\mathbf{Y}_t,\mathbf{Z}_t,\boldsymbol{\Theta}) = \mathbf{Y}_t - \mathbf{G}(\mathbf{X}_t,\mathbf{Z}_t,\boldsymbol{\Theta}).$$

Thus, the stochastic normal model (2.1)–(2.2) is derived from the deterministic functions $\mathbf{F}$ and $\mathbf{G}$ in (2.3)–(2.4).

A stochastic state space model is defined in general by three probability distributions

$$(2.5)\quad \begin{aligned} &(a)\ P(\mathbf{X}_1|\mathbf{Z}_1,\boldsymbol{\Theta}) \\ &(b)\ P(\mathbf{X}_t|\mathbf{X}_{t-1},\mathbf{Z}_t,\boldsymbol{\Theta}) \\ &(c)\ P(\mathbf{Y}_t|\mathbf{X}_t,\mathbf{Z}_t,\boldsymbol{\Theta}) \end{aligned}$$

which correspond, respectively, to an initial condition, a stochastic process equation, and a stochastic observation equation. Model (2.5), i.e., model G1 in Table 1, is the most general model discussed in this paper; all others can be considered special cases. Consequently, a statistical theory for (2.5) extends to the entire range of applications considered here. Two issues dominate this theory: simulating data from known parameters and estimating parameters from observed data. For convenience in the discussion, let $\mathbf{X}^t = \{\mathbf{X}_s\}_{s=1}^t$ denote the cumulative vector of states up to time $t$, with similar definitions for $\mathbf{Y}^t$ and $\mathbf{Z}^t$.

Simulation from (2.5) proceeds as follows. First, specify a parameter vector $\boldsymbol{\Theta}$ and controls $\mathbf{Z}^N$ up to time $t = N$. Second, use the unconditional distribution (2.5a) to generate an initial random state vector $\mathbf{X}_1$. Third, iteratively apply the conditional distribution (2.5b) to generate the random states $\mathbf{X}_t$, $t = 2,\ldots,N$, and thus complete the simulation of the cumulative state vector $\mathbf{X}^N$. Finally, use the conditional

TABLE 3. Notation for means and variances of the random variables $\mathbf{X}_t$, $\mathbf{Y}_t$, and $\mathbf{X}_t|\mathbf{Y}_t$, each conditioned on $\mathbf{Y}^{t-1}$.

| Variate | Mean | Variance |
|---|---|---|
| $\mathbf{X}_t\|\mathbf{Y}^{t-1}$ | $\mathbf{M}_{Xt}$ | $\mathbf{W}_{Xt}$ |
| $\mathbf{Y}_t\|\mathbf{Y}^{t-1}$ | $\mathbf{M}_{Yt}$ | $\mathbf{W}_{Yt}$ |
| $\mathbf{X}_t\|\mathbf{Y}^{t}$ | $\mathbf{M}^*_{Xt}$ | $\mathbf{W}^*_{Xt}$ |

distribution (2.5c) to generate random observations $\mathbf{Y}^N$ from the simulated states $\mathbf{X}^N$.

To use (2.5) for parameter estimation, consider first the situation in which *both* $\mathbf{X}_t$ and $\mathbf{Y}_t$ are observed up to time $t = N$. The likelihood for the complete data set $(\mathbf{X}^N,\mathbf{Y}^N)$ is then

$$(2.6) \qquad L(\mathbf{X}^N,\mathbf{Y}^N|\mathbf{Z}^N,\mathbf{\Theta}) = L(\mathbf{Y}^N|\mathbf{X}^N,\mathbf{Z}^N,\mathbf{\Theta})\, L(\mathbf{X}^N|\mathbf{Z}^N,\mathbf{\Theta}),$$

where

$$(2.7) \qquad L(\mathbf{Y}^N|\mathbf{X}^N,\mathbf{Z}^N,\mathbf{\Theta}) = \prod_{t=1}^{N} P(\mathbf{Y}_t|\mathbf{X}_t,\mathbf{Z}_t,\mathbf{\Theta})$$

$$(2.8) \qquad L(\mathbf{X}^N|\mathbf{Z}^N,\mathbf{\Theta}) = P(\mathbf{X}_1|\mathbf{Z}_1,\mathbf{\Theta}) \prod_{t=2}^{N} P(\mathbf{X}_t|\mathbf{X}_{t-1},\mathbf{Z}_t,\mathbf{\Theta}).$$

The calculation (2.6)–(2.8) illustrates an essential feature of the stochastic model (2.5): the overall likelihood can be expressed as a product of conditional probabilities. If both $\mathbf{X}^N$ and $\mathbf{Y}^N$ were observed, as well as the controls $\mathbf{Z}^N$, the likelihood (2.6) could be used for inferences about the parameter vector $\mathbf{\Theta}$.

In practice, $\mathbf{X}^N$ is not observed, and the distribution of observations $\mathbf{Y}^N$ can be obtained by integrating (2.6) across all possible values $\mathbf{X}^N$:

$$(2.9) \qquad L(\mathbf{Y}^N|\mathbf{Z}^N,\mathbf{\Theta}) = \int_{\mathbf{X}^N} L(\mathbf{Y}^N|\mathbf{X}^N,\mathbf{Z}^N,\mathbf{\Theta})\, L(\mathbf{X}^N|\mathbf{Z}^N,\mathbf{\Theta})\, d\mathbf{X}^N.$$

Thus, given the model (2.5) and the observations $\mathbf{Y}^N$, (2.9) defines the likelihood function required for statistical inferences about $\mathbf{\Theta}$. The discussion of state space model likelihoods could theoretically end here, except for two technical difficulties. First, the integral in (2.9) may pose a complex computational problem because $\mathbf{X}^N$ is a vector with a large number $(mN)$ of dimensions. Second, the unknown states $\mathbf{X}^N$ may have as much biological importance as the unknown parameters $\mathbf{\Theta}$.

To some extent, both difficulties can be addressed by shifting to a Bayes perspective. Suppose that the complete parameter vector

$$(2.10) \qquad \mathbf{\Psi} = (\mathbf{X}^N,\mathbf{\Theta})$$

includes all unknown states. Then a posterior distribution for $\mathbf{\Psi}$ can be defined by the proportionality

$$(2.11) \qquad P(\mathbf{X}^N,\mathbf{\Theta}|\mathbf{Y}^N,\mathbf{Z}^N) \propto L(\mathbf{Y}^N|\mathbf{X}^N,\mathbf{Z}^N,\mathbf{\Theta})\, L(\mathbf{X}^N|\mathbf{Z}^N,\mathbf{\Theta}) P(\mathbf{\Theta}).$$

In (2.11) the likelihood for the observations $\mathbf{Y}^N$, given the parameters $\mathbf{\Psi}$, is defined by (2.7); the prior for $\mathbf{X}^N$ is chosen to be the distribution (2.8); and $P(\mathbf{\Theta})$ denotes the prior for $\mathbf{\Theta}$. Thus, within the Bayes framework, the process distribution (2.5b) defines the *prior* (2.8) for the state parameters $\mathbf{X}^N$. Similarly, the distribution (2.5c) of observations defines the *likelihood* (2.7) for $\mathbf{Y}^N|\mathbf{\Psi}$. Except for the prior $P(\mathbf{\Theta})$, the right-hand sides of (2.6) and (2.11) are identical.

If the states $\mathbf{X}^N$ are considered nuisance parameters in (2.10), then the Bayes marginal distribution

$$(2.12) \qquad P(\mathbf{\Theta}|\mathbf{Y}^N,\mathbf{Z}^N) \propto \left(\int_{\mathbf{X}^N} L(\mathbf{Y}^N|\mathbf{X}^N,\mathbf{Z}^N,\mathbf{\Theta})\, L(\mathbf{X}^N|\mathbf{Z}^N,\mathbf{\Theta})\, d\mathbf{X}^N\right) P(\mathbf{\Theta})$$

corresponds to the likelihood (2.9). Thus, both frequentist and Bayes analyses ultimately lead to the integral in (2.9) or (2.12). Nevertheless, the Bayes framework easily allows the random states $\mathbf{X}_t$ to be considered parameters, so that the posterior (2.11) is meaningful without integration. By contrast, a frequentist interpretation of the likelihood (2.6) is more awkward; from this point of view, the states $\mathbf{X}_t$ are unobserved random variables, and (2.6) is not a classical likelihood function.

## 3. Estimation: KF Paradigm

For the linear normal model, the Kalman filter (KF) algorithm provides a means of calculating the integral (2.9) analytically. The algorithm uses a representation of the likelihood (2.9) that focuses exclusively on the observed data. Mathematically the distribution of $\mathbf{Y}^N$ depends entirely on $\mathbf{\Theta}$, and the states $\mathbf{X}_t$ $(t = 1,\ldots,N)$ act as artifacts in constructing this distribution. Thus, the likelihood (2.9) for $\mathbf{Y}^N$ can also be expressed as a product of conditional probabilities.

$$(3.1) \qquad L(\mathbf{Y}^N|\mathbf{\Theta}) = L(\mathbf{Y}_1|\mathbf{\Theta}) \prod_{t=2}^{N} L(\mathbf{Y}_t|\mathbf{Y}^{t-1},\mathbf{\Theta}),$$

where dependence on known control vectors $\mathbf{Z}_t$ is suppressed for simplicity.

The KF approach to calculating (3.1) involves relationships among the three conditional random variables listed in Table 3, along with notation for their means and variances. Starting from the data $\mathbf{Y}^{t-1}$ known at time $t - 1$, $\mathbf{X}_t|\mathbf{Y}^{t-1}$ and $\mathbf{Y}_t|\mathbf{Y}^{t-1}$ correspond to the predicted next state and observation, respectively. The third variate in Table 3

$$(3.2) \qquad \mathbf{X}_t|\mathbf{Y}^t = (\mathbf{X}_t|\mathbf{Y}_t)|\mathbf{Y}^{t-1}$$

pertains to the current state $\mathbf{X}_t$ estimated from past data $\mathbf{Y}^{t-1}$ and the current observation $\mathbf{Y}_t$. The Kalman filter was designed originally to provide estimates of the current state, based on current observations, as in (3.2). By contrast, the likelihood (3.1) depends on the second variate in Table 3, i.e., $\mathbf{Y}_t|\mathbf{Y}^{t-1}$.

Table 4 lists exact recursive equations for the means and variances in Table 3, based on the linear normal model (1.7)–(1.9). Their proof is remarkably simple. Because the model is linear with additive normal error, all conditional and unconditional variates generated by the model are multinormal. The inductive proof of Table 4 begins with the variate $\mathbf{X}_{t-1}|\mathbf{Y}^{t-1}$, which has a mean $\mathbf{M}^*_{X,t-1}$ and covariance matrix $\mathbf{W}^*_{X,t-1}$. The linear relationship (1.7) transforms this variate to $\mathbf{X}_t|\mathbf{Y}^{t-1}$ with mean (KL.1) and covariance matrix (KL.2). Thus, the mean is transformed by the linear relationship itself, and the covariance matrix is the sum of the transformed matrix $\mathbf{B}_t\mathbf{W}^*_{X,t-1}\mathbf{B}_t'$ plus the matrix $\mathbf{U}_t$ due to

TABLE 4. Recursive equations for means and variances of the random variables in Table 3, based on the linear normal model (1.7)–(1.9). Equation labels "KL" indicate the Kalman filter approach to the linear model. The prime symbol (′) and exponent "−1" denote the transpose and inverse, respectively, of a matrix.

| | |
|---|---|
| (KL.1) | $\mathbf{M}_{Xt} = \mathbf{A}_t + \mathbf{B}_t\mathbf{M}^*_{X,t-1}$ |
| (KL.2) | $\mathbf{W}_{Xt} = \mathbf{B}_t\,\mathbf{W}^*_{X,t-1}\mathbf{B}'_t + \mathbf{U}_t$ |
| (KL.3) | $\mathbf{M}_{Yt} = \mathbf{C}_t + \mathbf{D}_t\mathbf{M}_{Xt}$ |
| (KL.4) | $\mathbf{W}_{Yt} = \mathbf{D}_t\mathbf{W}_{Xt}\mathbf{D}'_t + \mathbf{V}_t$ |
| (KL.5) | $\mathbf{M}^*_{Xt} = \mathbf{M}_{Xt} + \mathbf{W}_{Xt}\mathbf{D}'_t(\mathbf{W}_{Yt})^{-1}(\mathbf{Y}_t - \mathbf{M}_{Yt})$ |
| (KL.6) | $\mathbf{W}^*_{Xt} = \mathbf{W}_{Xt} - \mathbf{W}_{Xt}\mathbf{D}'_t(\mathbf{W}_{Yt})^{-1}\,\mathbf{D}_t\mathbf{W}_{Xt}$ |

independent process error. A similar proof applies to (KL.3)–(KL.4), based on the second linear transformation (1.8). Finally, from the distributions just obtained for $\mathbf{X}_t$ and $\mathbf{Y}_t$, conditional on $\mathbf{Y}^{t-1}$, it is possible to derive the conditional distribution of $\mathbf{X}_t|\mathbf{Y}^t$. As discussed by Meinhold and Singpurwalla (1983), the analysis is similar to linear regression of $\mathbf{X}_t$ on $\mathbf{Y}_t$. Thus, in (KL.5), the mean $\mathbf{M}^*_{Xt}$ of $\mathbf{X}_t|\mathbf{Y}_t$ depends linearly on $\mathbf{Y}_t$. Appendix A completes the proof of (KL.5)–(KL.6).

From a computational viewpoint, the six equations in Table 4 recursively update three pairs of means and variances: $(\mathbf{M}_{Xt},\mathbf{W}_{Xt})$, $(\mathbf{M}_{Yt},\mathbf{W}_{Yt})$, and $(\mathbf{M}^*_{Xt},\mathbf{W}^*_{Xt})$. The model components $\mathbf{A}_t$, $\mathbf{B}_t$, $\mathbf{C}_t$, and $\mathbf{D}_t$ are presumed known, along with the data $\mathbf{Y}_t$. Equations (KL.1)–(KL.6) provide the update

$$(3.3) \qquad (\mathbf{M}^*_{X,t-1},\mathbf{W}^*_{X,t-1}) \rightarrow (\mathbf{M}^*_{Xt},\mathbf{W}^*_{Xt}).$$

Similarly, (KL.3)–(KL.6) and (KL.1)–(KL.2) sequentially give

$$(3.4) \qquad (\mathbf{M}_{Xt},\mathbf{W}_{Xt}) \rightarrow (\mathbf{M}_{X,t+1},\mathbf{W}_{X,t+1}).$$

Both (3.3) and (3.4) use all six equations and provide recursive estimates of all three mean–variance pairs.

The likelihood (3.1) for the linear normal model can be written analytically as the product of multinormal distributions

$$(3.5) \qquad L(\mathbf{Y}^N|\boldsymbol{\Theta}) = \left(\sqrt{2\pi}\right)^{-nN}$$

$$\times\prod_{t=1}^{N}|\,\mathbf{W}_{Yt}|^{-\frac{1}{2}}\exp\left[-\frac{1}{2}(\mathbf{Y}_t - \mathbf{M}_{Yt})'(\mathbf{W}_{Yt})^{-1}(\mathbf{Y}_t - \mathbf{M}_{Yt})\right],$$

where it is understood that $\mathbf{M}_{Yt}$ and $\mathbf{W}_{Yt}$ depend on $\boldsymbol{\Theta}$. The factor corresponding to $t = 1$ in (3.5) theoretically refers to the unconditional distribution of $\mathbf{Y}_1$; therefore, $\mathbf{M}_{Y1}$ and $\mathbf{W}_{Y1}$ require special definition. Usually, $\boldsymbol{\Theta}$ parametrically determines $\mathbf{M}_{X1}$ and $\mathbf{W}_{X1}$, the unconditional mean and variance of the initial state $\mathbf{X}_1$; these, in turn, determine $\mathbf{M}_{Y1}$ and $\mathbf{W}_{Y1}$ via (KL.3)–(KL.4). The likelihood for model (1.7)–(1.9), i.e., G4 in Table 1, is then completely defined by the recursion (3.4) in Table 4 and the function (3.5).

TABLE 5. Approximate recursive equations for means and variances of the random variables in Table 3, based on the nonlinear normal model (1.3)–(1.6). Equation labels "KN" indicate the Kalman filter approach to the nonlinear model. The symbol $\nabla_{\mathbf{X}}$ denotes the gradient with respect to $\mathbf{X}$.

| | |
|---|---|
| (KN.1) | $\mathbf{M}_{Xt} = \mathbf{F}(\mathbf{M}^*_{X,t-1},\mathbf{Z}_t,\boldsymbol{\Theta})$ |
| (KN.2a) | $\mathbf{B}_t = \nabla_{\mathbf{X}}\mathbf{F}(\mathbf{M}^*_{X,t-1},\mathbf{Z}_t,\boldsymbol{\Theta})$ |
| (KN.2b) | $\mathbf{W}_{Xt} = \mathbf{B}_t\mathbf{W}^*_{X,t-1}\mathbf{B}'_t + \mathbf{U}_t$ |
| (KN.3) | $\mathbf{M}_{Yt} = \mathbf{G}(\mathbf{M}_{Xt},\mathbf{Z}_t,\boldsymbol{\Theta})$ |
| (KN.4a) | $\mathbf{D}_t = \nabla_{\mathbf{X}}\mathbf{G}(\mathbf{M}_{Xt},\mathbf{Z}_t,\boldsymbol{\Theta})$ |
| (KN.4b) | $\mathbf{W}_{Yt} = \mathbf{D}_t\mathbf{W}_{Xt}\mathbf{D}'_t + \mathbf{V}_t$ |
| (KN.5) | $\mathbf{M}^*_{Xt} = \mathbf{M}_{Xt} + \mathbf{W}_{Xt}\mathbf{D}'_t(\mathbf{W}_{Yt})^{-1}(\mathbf{Y}_t - \mathbf{M}_{Yt})$ |
| (KN.6) | $\mathbf{W}^*_{Xt} = \mathbf{W}_{Xt} - \mathbf{W}_{Xt}\mathbf{D}'_t(\mathbf{W}_{Yt})^{-1}\,\mathbf{D}_t\mathbf{W}_{Xt}$ |

Table 5 shows a similar calculation that can be used to approximate the likelihood function for the (possibly nonlinear) normal model (1.3)–(1.6), i.e., G2 in Table 1. Two features distinguish the KF algorithm in Table 4 and from the "extended" Kalman filter in Table 5. First, the linear update equations (KL.1) and (KL.3) are replaced by their nonlinear counterparts (KN.1) and (KN.3). Second, equations (KN.2a) and (KN.4a) are added to Table 5. These use gradients of the vector functions $\mathbf{F}$ and $\mathbf{G}$ to define matrices analogous to $\mathbf{B}_t$ and $\mathbf{D}_t$ in Table 4. For example, if $\mathbf{X} = (X_1,\ldots,X_m)$ and $\mathbf{F}(\mathbf{X}) = (F_1(\mathbf{X}),\ldots,F_m(\mathbf{X}))$, then

$$(\nabla_{\mathbf{X}}\mathbf{F}(\mathbf{X}))_{ij} = \frac{\partial F_i\,(\mathbf{X})}{\partial X_j}$$

defines the element in position $(i,j)$ of the $m \times m$ matrix $\nabla_{\mathbf{X}}\mathbf{F}$. Similarly, $\nabla_{\mathbf{X}}\mathbf{G}$ has dimensions $n \times m$. Conceptually, Tables 4 and 5 have the same practical purpose: calculation of the likelihood (3.5) via recursive updates (3.4). Table 5 reduces to Table 4 when $\mathbf{F}$ and $\mathbf{G}$ are linear functions of $\mathbf{X}$.

The recursions in Table 5 are based on three assumptions. First, at each time step, the distributions of the variates in Table 3 are approximately normal, comparable with the exact normal distributions in the linear model. Second, means can be updated using the deterministic nonlinear equations (1.1)–(1.2). Finally, variance around the mean is small enough that variance can be updated using the linear formulas (KN.2b) and (KN.4b), which are identical to (KL.2) and (KL.4), respectively. Essentially, Table 5 assumes that trajectories of states and observations obey the full nonlinear model (1.1)–(1.2); however, noise is small enough that linear approximations can be used to update variances. The analysis does *not*, however, merely replace the nonlinear model with a first-order linear approximation. For example, as model noise is reduced to zero (i.e., $\mathbf{U}_t \rightarrow 0$ and $\mathbf{V}_t \rightarrow 0$ in (1.3)–(1.4)), the recursions in Table 5 converge to exact nonlinear trajectories.

The linear normal model (1.10)–(1.12) provides a simple illustration of the concepts developed here. The definitions (1.13)–(1.15) provide all the information required for the recursions in Table 4, given the observations $\mathbf{Y}_t$. Thus, the likelihood function (3.5) is completely defined, once expressions are given for the unconditional mean and variance $\mathbf{M}_{X1}$ and $\mathbf{W}_{X1}$. For simplicity, use the parameter vector (1.16) to define

$$(3.6) \qquad \mathbf{M}_{X1} = \begin{pmatrix} R_1 \\ S_1 \end{pmatrix}, \mathbf{W}_{X1} = \begin{pmatrix} \sigma_1^2 & 0 \\ 0 & \sigma_2^2 \end{pmatrix}.$$

TABLE 6. Recursive equations for quantities $(\tilde{\boldsymbol{\delta}}_t, \tilde{\mathbf{X}}_t, \tilde{\boldsymbol{\epsilon}}_t)$, $t = 3,\ldots,N$, used in calculating the state vector estimate (4.4). Equation labels "EV" indicate the errors in variables estimation method. The recursions apply to both linear and nonlinear normal models (1.7)–(1.9) and (1.3)–(1.6), although equations (EV.1)–(EV.2) are redundant to the linear model definition.

| | |
|---|---|
| (EV.1) | $\mathbf{B}_t = \nabla_{\mathbf{X}}\mathbf{F}(\tilde{\mathbf{X}}_{t-1},\mathbf{Z}_t,\boldsymbol{\Theta})$ |
| (EV.2) | $\mathbf{D}_{t-1} = \nabla_{\mathbf{X}}\mathbf{G}(\tilde{\mathbf{X}}_{t-1},\mathbf{Z}_{t-1},\boldsymbol{\Theta})$ |
| (EV.3) | $\tilde{\boldsymbol{\delta}}_t = \mathbf{U}_t(\mathbf{B}_t^{-1})'[\mathbf{U}_{t-1}^{-1}\tilde{\boldsymbol{\delta}}_{t-1} - \mathbf{D}_{t-1}\mathbf{V}_{t-1}^{-1}\tilde{\boldsymbol{\epsilon}}_{t-1}]$ |
| (EV.4) | $\tilde{\mathbf{X}}_t = F(\tilde{\mathbf{X}}_{t-1},\mathbf{Z}_t,\boldsymbol{\Theta}) + \tilde{\boldsymbol{\delta}}_t$ |
| (EV.5) | $\tilde{\boldsymbol{\epsilon}}_t = \mathbf{Y}_t - \mathbf{G}(\tilde{\mathbf{X}}_t,\mathbf{Z}_t,\boldsymbol{\Theta})$ |

The initialization (3.6) interprets the parameters $R_1$ and $S_1$ as unconditional means and uses the conditional variance $\mathbf{U}_1$ to estimate the unconditional variance $\mathbf{W}_{X1}$. Although (3.6) may underestimate initial variance components, the recursion (3.4) in Table 4 typically adjusts $\mathbf{W}_{Xt}$ to realistic values after a few iterations. Furthermore, the likelihood (3.5) tends to be insensitive to reasonable parametric choices for $\mathbf{W}_{X1}$.

## 4. Estimation: EV Paradigm

The errors in variables (EV) paradigm treats unobserved states $\mathbf{X}_t$ as parameters, rather than random variables. Statistical inference is based on the likelihood (2.6) or the Bayes posterior (2.11), rather than the integral (2.9) or (2.12). As discussed in Section 2, this approach can be justified rigorously from a Bayes viewpoint, although it is somewhat ad hoc from a frequentist perspective.

The complete parameter vector $\boldsymbol{\Psi}$ in (2.10) includes both states $\mathbf{X}^N$ and conventional parameters $\boldsymbol{\Theta}$. These may be somewhat redundant; typically, $\boldsymbol{\Theta}$ determines the $m$ components of $\mathbf{X}_1$, as in (1.16). Thus, although the KF paradigm requires estimating only the $k$ components of $\boldsymbol{\Theta}$, the EV paradigm requires estimates for an additional $m(N - 1)$ components of $\mathbf{X}^N$. EV estimation is inherently high dimensional because the number of parameters increases linearly with the number $N$ of time periods.

From a frequentist perspective, the EV paradigm requires computing maximum likelihood (ML) estimates $\hat{\boldsymbol{\Psi}}$ from (2.6). Similarly, Bayes EV estimation requires highest posterior density (HPD) estimates from (2.11). In principle, a general procedure to maximize functions can be used to solve all estimation problems described in this paper (KF or EV; frequentist or Bayes). The selected approach determines the function to be maximized and the dimensionality of the parameter vector.

The distinction between KF and EV paradigms becomes particularly clear in the Bayes context. Within the KF framework, the recursions in Tables 4 and 5 yield the Bayes mean

(4.1) $\mathbf{M}_{Xt}^* = E[\mathbf{X}_t|\mathbf{Y}^t]$

for each $t$. Within the EV framework, if the Bayes posterior is multinormal, then an HPD parameter estimate is also the mean value of that parameter, given all available data; thus,

(4.2) $\hat{\mathbf{X}}_t = E[\mathbf{X}_t|\mathbf{Y}^N]$.

For the linear normal model, (2.12) is multinormal in $\mathbf{X}^N$ and (4.2) is exact; otherwise, (4.2) is approximate.

Comparing (4.1) with (4.2) shows that the KF paradigm gives *current* state estimates, based on data up to the current time $t$. This is evident computationally in Tables 4 and 5, where $\mathbf{M}_{Xt}^*$ depends recursively only on data up to time $t$. By contrast, the EV paradigm gives *retrospective* state estimates, in which all data up to time $t = N$ influence the estimate at each time $t$. These results apply to a specified parameter vector $\boldsymbol{\Theta}$. When $\boldsymbol{\Theta}$ is estimated, $\mathbf{M}_{Xt}^*$ in (4.1) depends indirectly on $\mathbf{Y}^N$ through the estimate $\hat{\boldsymbol{\Theta}}$.

The high dimensionality of EV estimation can sometimes be reduced by taking advantage of relationships among the estimates $\hat{\mathbf{X}}_t$. For example, the gradient equations

(4.3) $\nabla\mathbf{x}_t P(\mathbf{X}^N,\boldsymbol{\Theta}|\mathbf{Y}^N,\mathbf{Z}^N) = 0$

obtained from the posterior (2.11) produce a system of $N - 1$ linear equations in the $N - 1$ unknowns $\mathbf{X}_2,\ldots, \mathbf{X}_N$. Theoretically, these equations determine the HPD estimates $\hat{\mathbf{X}}_2,\ldots, \hat{\mathbf{X}}_N$.

Table 6 provides an algorithm for computing these estimates for the normal model (1.3)–(1.6). Suppose that $\boldsymbol{\Theta}$ is given, $\mathbf{X}_1$ is determined by $\boldsymbol{\Theta}$, and a trial value $\mathbf{X}_2$ is selected. Using these values, define $\boldsymbol{\delta}_2$ and $\boldsymbol{\epsilon}_2$ from (2.3)–(2.4). Apply (EV.1)–(EV.5) recursively with $t = 3,\ldots, N$ to obtain the estimated state vector

(4.4) $\tilde{\mathbf{X}}^N = (\mathbf{X}_1,\mathbf{X}_2,\tilde{\mathbf{X}}_3,\ldots, \tilde{\mathbf{X}}_N)$

conditional on $\mathbf{X}_1$ (from $\boldsymbol{\Theta}$) and the trial value $\mathbf{X}_2$. Define

(4.5) $\tilde{L}(\boldsymbol{\Theta},\mathbf{X}_2) = L(\mathbf{Y}^N|\tilde{\mathbf{X}}^N,\boldsymbol{\Theta})\ L(\tilde{\mathbf{X}}^N|\boldsymbol{\Theta})$

and compute the vector $\tilde{\mathbf{X}}_2$ that maximizes $\tilde{L}$. Then it can be proved (Appendix B) that the vector (4.4) corresponding to $\tilde{\mathbf{X}}_2$

$$\tilde{\mathbf{X}}^N = (\mathbf{X}_1,\tilde{\mathbf{X}}_2,\tilde{\mathbf{X}}_3,\ldots, \tilde{\mathbf{X}}_N)$$

is both an HPD and ML estimate of $\mathbf{X}^N$, conditional on $\mathbf{X}_1$. Computationally, maximizing (4.5) across one vector $\mathbf{X}_2$ may be more efficient than maximizing the likelihood (2.6) or the posterior (2.11) across $N - 1$ vectors $\mathbf{X}_t$ ($t = 2,\ldots, N$).

Incidentally, $\tilde{\mathbf{X}}^N$ in (4.4) is *not* HPD for arbitrary given vectors $\mathbf{X}_1$ and $\mathbf{X}_2$. The recursions in Table 6 fail to take account of (4.3) with $t = N$. This condition is satisfied only when $\mathbf{X}_2$ is assigned the value $\tilde{\mathbf{X}}_2$ that maximizes (4.5). Thus, although $\tilde{\mathbf{X}}_t$ in Table 6 depends only on past data, maximization in (4.5) forces the final HPD estimates $\tilde{\mathbf{X}}_t$ to depend on the entire data series $\mathbf{Y}^N$, as indicated in (4.2). For the linear normal model, a series of forward and backward recursions can be used to replace the maximization across $\mathbf{X}_2$. These resemble an iterative method proposed by Box and Jenkins (1976, chap 7.1) for computing autoregressive moving average (ARMA) model likelihoods.

The KF recursions in Tables 4 and 5 are somewhat more robust than the EV recursions in Table 6. For example, the KF paradigm requires an invertible matrix $\mathbf{W}_{Yt}$ in (KL.5)–(KL.6) or (KN.5)–(KN.6). By contrast, (EV.3) requires that the matrices $\mathbf{B}_t$, $\mathbf{U}_t$ and $\mathbf{V}_t$ should all have inverses. If they do not, the maximum (4.5) may still exist; however, it cannot be computed via the recursions in Table 6.

The EV recursions can, for example, be applied to the linear model (1.10)–(1.12) if $\sigma_1$, $\sigma_2$, $\tau$, and $s$ are greater than 0. In particular, the required inverse of $\mathbf{B}_t$ in (1.14) is

$$\mathbf{B}_t^{-1} = \begin{pmatrix} 1 & 0 \\ -1 & s^{-1} \end{pmatrix}.$$

A discussion of the EV paradigm would be incomplete without some words of caution. Although the method is rigorous from a Bayes viewpoint, even a Bayes analyst might argue that HPD estimates are inappropriate. I have pointed out that (4.2) is only approximate when the posterior is not multinormal. Perhaps $E[\mathbf{X}_t|\mathbf{Y}^N]$ provides a better estimator of $\mathbf{X}_t$ than the HPD estimate $\hat{\mathbf{X}}_t$. Furthermore, the marginal posterior distribution $P(\mathbf{X}_t|\mathbf{Y}^N)$ is preferable to a single estimate because it summarizes all information available from the data $\mathbf{Y}^N$ about the state vector $\mathbf{X}_t$. Unfortunately, high dimensional numeric integration is required in general to compute either $E[\mathbf{X}_t|\mathbf{Y}^N]$ or $P(\mathbf{X}_t|\mathbf{Y}^N)$ from $P(\mathbf{X}^N,\mathbf{\Theta}|\mathbf{Y}^N)$ in (2.11).

Classically, the unknown states $\mathbf{X}_t$ constitute missing data, rather than parameters. The KF paradigm provides a computational means of handling the "expectation" step within the EM algorithm proposed by Dempster et al. (1977) for dealing with missing data. The EV paradigm avoids this step by maximizing across both parameters and unknown states. Little and Rubin (1987, p. 92) examine this strict maximization approach in the context of sampling theory. They conclude that "Although useful in particular problems, this is not generally a reliable approach to the analysis of incomplete data." Mendelssohn (1988) reinforces their conclusions in the context of a model for catch and age data. I discuss this point further in Section 6.

## 5. Model Design

Model (1.10)–(1.12) illustrates state space methodology in a relatively simple fisheries context. As a more complex example, consider an extension of this model to include catch-at-age data. Three questions must be addressed in designing any state space model. First, what are the components of the state vector $\mathbf{X}_t$, the observation vector $\mathbf{Y}_t$, and the control vector $\mathbf{Z}_t$? Second, what functions $\mathbf{F}$ and $\mathbf{G}$ define the process and observation equations (1.1)–(1.2), and what parameters $\mathbf{\Theta}$ do these functions require? Finally, how is stochastic error imposed on these equations; more precisely, what are the distributions (2.5), and what additional parameters $\mathbf{\Theta}$ do they introduce?

### 5A. Question 1: Vector Definitions

Usually $\mathbf{Y}_t$ and $\mathbf{Z}_t$ are easiest to define because they correspond to data actually available for the analysis. Some data, however, may ambiguously be included in either $\mathbf{Y}_t$ or $\mathbf{Z}_t$. For example, if the catch $C_t$ is measured with error, it may be necessary to distinguish the observed catch $C_t$ from a hidden state variable $D_t$ that corresponds to the actual catch. Thus, the term "control" needs to be interpreted in the mathematical context set out in (1.1)–(1.2) and (2.5). Although the true catch $D_t$ is a control in the conventional sense that it resulted from human intervention, its actual value may have to be estimated along with other state variables.

In the example here, I assume that the following data are available for each year $t = 1,\ldots,N$: (1) the catch biomass $C_t$, (2) a biomass index $I_t$, (3) the observed proportion $p_{at}$ of fish in age class $a = 1,\ldots,A$, and (4) the sample size $h_t$ of aged fish structures. Two additional data sources are presumed available, independent of year $t$: (5) the mean weight $w_a$ of fish in age class $a$ and (6) the classification matrix

(5.1) $$\Pi = \{\pi_{ab}\}_{a,b=1}^{A},$$

where $\pi_{ab}$ denotes the probability that a reader will classify a fish in age class $a$ when its true age class is $b$. In this discussion, "age class" and "age" need not be the same. Typically, age class 1 corresponds to the age of first recruitment, and the final age class $A$ refers to all ages above some specified age. The sample size $h_t$ is not essential; more generally, $h_t$ can be any relative measure of sample accuracy in year $t$, where it is possible that $h_t$ is a constant $h$. The known weights $w_a$ can easily be generalized to weights $w_{at}$ that vary annually, if such data are available. I assume that the matrix (5.1) has been estimated from reader experiments, as described by Richards et al. (1992). The probability definition of $\mathbf{\Pi}$ implies that

(5.2) $$\sum_{a=1}^{A} \pi_{ab} = 1$$

for each true age $b$. If fish are aged perfectly, then $\mathbf{\Pi}$ is the identity matrix.

The method of data collection influences the design of a state space model. Here I assume that (1) $C_t$ is collected from commercial records, such as sales slips, (2) $I_t$ comes from independent sources, such as CPUE from research surveys or logbooks from individual commercial vessels, and (3) $p_{at}$ is compiled from random samples of the commercial catch, subsequently aged by experienced readers. Thus, data sources (1), (2), and (3) are independent. By contrast, the theoretical biomass of fish caught at age $a$ in year $t$

(5.3) $$C_{at} = \frac{w_a p_{at}}{\Sigma_{a=1}^{A} w_a p_{at}} C_t$$

is a composite measurement that is not part of the observation vector.

In many fisheries, the catch biomass $C_t$ is relatively well known. It may be biased low, due to unreported catch, and this systematic error could be built into the observation equation if the bias factor were known. Here I assume that $C_t$ can be treated as part of the control vector, as in (1.13), and that

(5.4) $$\mathbf{Y}_t = \begin{pmatrix} I_t \\ p_{2t} \\ \vdots \\ p_{At} \end{pmatrix}, \mathbf{Z}_t = \begin{pmatrix} C_t \\ C_{t-1} \\ h_t \end{pmatrix},$$

where $p_{1t}$ is defined from $\mathbf{Y}_t$ by

(5.5) $$p_{1t} = 1 - \sum_{a=2}^{A} p_{at}.$$

In principle, the vector of weights $w_a$ and the matrix $\mathbf{\Pi}$ should be included in $\mathbf{Z}_t$; however, because these do not depend on $t$, I treat them as known constants.

The definition of $\mathbf{X}_t$ depends on the resolution of the observed data. Because data are available by age class $a$, a logical choice for the state vector in this example is

(5.6) $$\mathbf{X}_t = \begin{pmatrix} K_{1t} \\ \vdots \\ K_{At} \end{pmatrix},$$

where $K_{at}$ denotes numbers of fish at age $a$ in year $t$.

TABLE 7. Deterministic equations defining the process function **F** and observation function **G** for a model with $\mathbf{X}_t$, $\mathbf{Y}_t$, and $\mathbf{Z}_t$ defined in (5.4) and (5.6). Equation labels "CA" indicate a catch-age-age model.

*Selectivity*

$$\text{(CA.1)} \quad \beta_a = \beta_1 + (1-\beta_1)\frac{1-\alpha^{a-1}}{1-\alpha^{A-1}}$$

*State moments*

$$\text{(CA.2)} \quad B_t(\mathbf{X}_t) = \sum_{a=1}^{A} w_a \beta_a K_{at}$$

$$\text{(CA.3)} \quad P_t(\mathbf{X}_t) = \sum_{a=1}^{A} \beta_a K_{at}$$

$$\text{(CA.4)} \quad u_{at}(\mathbf{X}_t) = \beta_a K_{at}/P_t(\mathbf{X}_t)$$

$$\text{(CA.5)} \quad v_{at}(\mathbf{X}_t) = \sum_{b=1}^{A} \pi_{ab} u_{bt}(\mathbf{X}_t)$$

$$\text{(CA.6)} \quad z_t(\mathbf{X}_t) = B_t(\mathbf{X}_t)/P_t(\mathbf{X}_t)$$

*Process function* **F**

$$\text{(CA.7)} \quad K_{1t} = K_{1,t-1}$$

$$\text{(CA.8)} \quad K_{at} = s\left[K_{a-1,t-1} - u_{a-1,t-1}\frac{C_{t-1}}{z_{t-1}}\right]; \qquad 2 \le a < A$$

$$\text{(CA.9)} \quad K_{At} = s\left[K_{A-1,t-1} + K_{A,t-1} - (u_{A-1,t-1} + u_{A,t-1})\frac{C_{t-1}}{z_{t-1}}\right]$$

*Observation function* **G**

$$\text{(CA.10)} \quad I_t = q(B_t - \frac{1}{2}C_t)$$

$$\text{(CA.11)} \quad p_{at} = v_{at}; \qquad 2 \le a \le A$$

## 5B. Question 2: System Equations

Table 7 presents deterministic functions **F** and **G** that define a catch-at-age model appropriate for the vectors (5.4) and (5.6). First, the relationship (CA.1) determines selectivities $\beta_a$ ($a = 1,\ldots,A$) from two parameters $\alpha$ and $\beta_1$, where $0 < \alpha < 1$ and $0 < \beta_1 \le 1$. For simplicity, the model here assumes that $\beta_a$ increases with age class $a$, with the final selectivity $\beta_A = 1$. Second, these selectivities and known fish weights $w_a$ determine population moments (CA.2)–(CA.6) as functions of the state vector $\mathbf{X}_t$. Conceptually, $B_t$ and $P_t$ represent the biomass and population available to the fishery, so that $z_t$ is the mean weight of available fish. The proportion of fish at age $a$ available to the fishery in year $t$ is $u_{at}$, where (CA.3)–(CA.4) imply that

$$\text{(5.7)} \quad \sum_{a=1}^{A} u_{at} = 1.$$

The apparent proportion $v_{at}$ of available fish is computed from the true proportions $u_{bt}$ and the reader classification matrix (5.1). It follows from (5.2), (5.7), and (CA.5) that

TABLE 8. Definitions used to extend the deterministic model in Table 7 to stochastic normal model. Equation numbers proceed sequentially from those in Table 7.

$$\text{(CA.12)} \quad \delta_{at} = \log(K_{at}) - \log(\overline{K_{at}})$$

$$\text{(CA.13)} \quad \epsilon_{It} = \log(I_t) - \log(\overline{I_t})$$

$$\text{(CA.14)} \quad \epsilon_{at} = \arcsin(\sqrt{p_{at}}) - \arcsin(\sqrt{\overline{p_{at}}}); \qquad 2 \le a \le A$$

$$\text{(CA.15)} \quad \mathbf{U}_t = \begin{pmatrix} \sigma_1^2 & \rho_1\sigma_1\sigma_2 & \rho_1\sigma_1\sigma_2 & \cdots & \rho_1\sigma_1\sigma_2 \\ \rho_1\sigma_1\sigma_2 & \sigma_2^2 & \rho_2\sigma_2^2 & \cdots & \rho_2\sigma_2^2 \\ \rho_1\sigma_1\sigma_2 & \rho_2\sigma_2^2 & \sigma_2^2 & \cdots & \rho_2\sigma_2^2 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \rho_1\sigma_1\sigma_2 & \rho_2\sigma_2^2 & \rho_2\sigma_2^2 & \cdots & \sigma_2^2 \end{pmatrix}$$

$$\text{(CA.16)} \quad \mathbf{V}_t = \begin{pmatrix} \tau_1^2 & 0 & 0 & \cdots & 0 \\ 0 & \tau_2^2/h_t & \rho_3\tau_2^2/h_t & \cdots & \rho_3\tau_2^2/h_t \\ 0 & \rho_3\tau_2^2/h_t & \tau_2^2/h_t & \cdots & \rho_3\tau_2^2/h_t \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & \rho_3\tau_2^2/h_t & \rho_3\tau_2^2/h_t & \cdots & \tau_2^2/h_t \end{pmatrix}$$

$$\text{(5.8)} \quad \sum_{a=1}^{A} v_{at} = 1.$$

The process function **F** can be expressed in terms of computed moments $u_{at}(\mathbf{X}_t)$ and $z_t(\mathbf{X}_t)$. The first component $\mathbf{X}_{1t}$ of the state vector represents recruitment, and (CA.7) is simply the deterministic counterpart of (1.10), i.e., constant recruitment. For $2 \le a < A$, (CA.8) expresses $K_{at}$ in terms of $K_{a-1,t-1}$, where $C_{t-1}/z_{t-1}$ is the number of fish removed in year $t-1$ and $u_{a-1,t-1}$ is the proportion of these fish available at age $a-1$. The natural survival $s = e^{-M}$ can alternatively be expressed in terms of natural mortality $M$. The process equation (CA.9) for the final component $K_{At}$ of the state vector is similar to (CA.8), except that both age classes $A-1$ and $A$ progress into age class $A$. Collectively, the $A$ equations (CA.7)–(CA.9) define the vector function $\mathbf{F}(\mathbf{X}_{t-1})$. Furthermore, the conservation relationship

$$\text{(5.9)} \quad K_t = K_{1t} + s\left(K_{t-1} - \frac{C_{t-1}}{z_{t-1}}\right)$$

follows from (5.7) and (CA.8)–(CA.9), where $K_t = \Sigma_{a=1}^{A} K_{at}$ is the total population at the start of year $t$.

The observation function **G** involves computed moments $B_t(\mathbf{X}_t)$ and $v_{at}(\mathbf{X}_t)$. Motivation for the index equation (CA.10) is similar to that discussed earlier for equation (1.12). In particular, (CA.10) assumes that $I_t$ indexes biomass available to the fishery, rather than total biomass. The remaining observation equations (CA.11) simply equate observed proportions $p_{at}$ to computed apparent proportions $v_{at}$ ($2 \le a \le A$). From (5.5) and (5.8), these imply that $p_{1t} = v_{1t}$. Collectively, the $A$ equations (CA.10)–(CA.11) define the vector function $\mathbf{G}(\mathbf{X}_t)$.

The model defined in Table 7 is generally nonlinear, due to ratios in (CA.4), (CA.6), and (CA.8)–(CA.9). Unlike most models in current use (e.g., Fournier and Archibald 1982; Deriso et al. 1985; Methot 1990), this one makes no reference to fishing mortalities and the Baronov catch equation (Ricker 1975, p. 13). For simplicity, the fishery is assumed to precede natural mortality and the catch biomass

$C_t$ is divided into age groups according to availability within the existing population. The model reduces to a deterministic version of (1.10)–(1.12) when $A = 2$, $w_a = w$, and $\beta_1 = 1$, that is, when there are only two age classes and both weights and selectivities are constant. Thus, in this special case, the model becomes linear.

### 5C. Question 3: Stochastic Error

Table 8 lists definitions that can be used to extend the deterministic model in Table 7 to a normal stochastic model. For simplicity, a line over a quantity is used to denote its predicted value. Thus, the prediction $\overline{K_{at}}$, which depends on the value of $a$, is calculated from the right-hand side of equation (CA.8) when $2 \le a < A$. Stochastic model distributions (2.1)–(2.2) are determined by residual vectors

$$\boldsymbol{\delta}_t = (\delta_{at})_{a=1}^{A},\ \boldsymbol{\epsilon}_t = (\epsilon_{at})_{a=1}^{A}$$

and covariance matrices $\mathbf{U}_t$ and $\mathbf{V}_t$ defined in Table 8. Transformations in (CA.12)–(CA.14) make this residual calculation different from (2.3)–(2.4). Strictly speaking, the model here is normal with respect to state and observation vectors

$$(5.10)\quad \mathbf{X}_t = \begin{pmatrix} \log(K_{1t}) \\ \vdots \\ \log(K_{At}) \end{pmatrix}, \mathbf{Y}_t = \begin{pmatrix} \log(I_t) \\ \arcsin(\sqrt{p_{2t}}) \\ \vdots \\ \arcsin(\sqrt{p_{At}}) \end{pmatrix},$$

redefined by transforming (5.4) and (5.6).

The complete parameter vector for the model in Tables 7 and 8 is

$$(5.11)\quad \boldsymbol{\Theta} = (\alpha, \beta_1, s, q, \rho_1, \rho_2, \rho_3, \sigma_1, \sigma_2, \tau_1, \tau_2, K_{11}, \ldots, K_{A1}).$$

Like (1.16), (5.11) can be divided into deterministic ($\alpha, \ldots, q$), stochastic ($\rho_1, \ldots, \tau_2$), and initializing ($K_{11}, \ldots, K_{A1}$) parameters. Here, $\sigma_1^2$ and $\sigma_2^2$ denote lognormal variances of recruitment and survival, respectively, where survival variance is presumed constant for all age classes. Survival and recruitment are correlated with coefficient $\rho_1$; similarly, survivals of different age classes have correlation $\rho_2$. Index observations $I_t$ have lognormal measurement error with variance $\tau_1^2$, where this error is assumed independent of measurement error in the proportions $p_{at}$. Furthermore, these proportions are presumed normalized by the "arcsin square root" transformation with (1) variance $\tau_2^2/h_t$ inversely proportional to the sample size $h_t$ and (2) interage correlation $\rho_3$. Finally, the parameters $K_{a1}$ in (5.11) and the matrix (CA.15) can be used to define the KF mean and covariance matrix of the initial distribution (2.5a), as in the discussion of (3.6). Within the EV paradigm, (2.5a) can be taken as any reasonable prior for the initial states $\log(K_{a1})$. If the prior is diffuse, then this factor is effectively dropped from the likelihood (2.8).

The normalized residual (CA.14) can be compared with earlier use of a multinomial likelihood function (Fournier and Archibald 1982) or lognormal error attached to the composite data (5.3) (Deriso et al. 1985). According to the multinomial distribution, $p_{at}$ should have mean and variance

$$(5.12)\quad E[p_{at}] = v_{at}, V[p_{at}] = \frac{v_{at}(1 - v_{at})}{h_t}.$$

The free parameter $\tau_2$ in (CA.16) gives the normalized model greater flexibility in the relationship between variance and sample size $h_t$. Furthermore, to illustrate a model designed around independent data sources, I have avoided the use of composite data, such as (5.3).

Simulation experiments indicate that the "arcsin square root" transformation has fairly robust ability to normalize estimated proportions $\hat{p} \ge 1\%$, based on samples of moderate size. Nevertheless, normal error appears unduly restrictive for a stochastic extension of the model in Table 7. Mathematically, the Dirichlet distribution (Kendall et al. 1987, p. 209) offers a more reasonable alternative to multivariate proportion data. From this point of view, the probability distribution for the proportions $p_{at}$ is

$$(5.13)\quad P(p_{2t}, \ldots, p_{At} \mid v_{2t}, \ldots, v_{At}) = \Gamma(\gamma h_t) \prod_{a=1}^{A} \frac{p_{at}^{\gamma h_t v_{at} - 1}}{\Gamma(\gamma h_t v_{at})},$$

where $\gamma$ is a variance parameter roughly analogous to $1/\tau_2^2$ in the normal model, $p_{1t}$ and $v_{1t}$ are calculated from (5.5) and (5.8), and $\Gamma$ refers to the gamma function. For a model based on (5.13), the distribution (2.5b) of observations is the product of the normal distribution associated with $\epsilon_{1t}$ in (CA.13) and the Dirichlet distribution (5.13).

Just as the multinomial can be regarded as a multivariate binomial distribution with moments (5.12), the Dirichlet can be considered a multivariate beta distribution in which

$$(5.14)\quad E[p_{at}] = v_{at}, V[p_{at}] = \frac{v_{at}(1 - v_{at})}{1 + \gamma h_t}.$$

Thus, as in the normalized model, an extra parameter ($\gamma$) is added to the variance formula (5.14). To simulate Dirichlet variates $p_{at}$, generate independent random numbers $x_{at}$ from the gamma distribution

$$x_{at} \sim G(x \mid \gamma h_t v_{at}) = \frac{1}{\Gamma(\gamma h_t v_{at})} x^{\gamma h_t v_{at} - 1} e^{-x}$$

and define $p_{at} = x_{at}/\sum_{a=1}^{A} x_{at}$.

## 6. Discussion

The catch-at-age model in Tables 7 and 8 illustrates the discipline and flexibility that state space methodology imposes on model design. The model itself is certainly not definitive; rather, its development shows how such models can be tailored to specific situations. The three questions cited at the start of Section 5 serve as guidelines for building any sequential fisheries model, including multispecies models or models with environmental controls.

Readers familiar with existing catch-at-age models may wonder why the parameter vector $\boldsymbol{\Theta}$ in (5.11) includes only an initial recruitment $K_{11}$, rather than recruitment $K_{1t}$ for all years $t = 1, \ldots, N$. In the deterministic case (CA.7) of constant recruitment, the restriction to a single parameter clearly makes sense. More generally, as $\sigma_1$ increases from 0, the stochastic counterpart of (CA.7) allows variable recruitment. As $\sigma_1$ becomes large, the states $K_{1t}$ become essentially independent of each other, as is often assumed in classical catch-at-age models.

Although the model here involves a survival parameter $s = e^{-M}$, where $M$ is natural mortality, it does not assume that natural mortality is constant. Lognormal stochastic versions of (CA.8)–(CA.9) include the expression

$$\log s + \delta_{at} = -M + \delta_{at}.$$

Thus, process error in the survival equations can be interpreted as variable natural mortality, a feature absent in many catch-at-age models. Another approach might be to include a survival $s_t$ in the state vector $\mathbf{X}_t$ and to augment the process function **F** with an equation that describes the sequential development of $s_t$. Like the recruitment example in the preceding paragraph, this alternative illustrates how the distinction between states and parameters can become blurred.

I have emphasized that the general stochastic model (2.5) can be used for both simulation and estimation. This is an important modeling requirement because an iterative cycle of simulation and estimation provides a test of how well parameters can actually be estimated. Reasonable algorithms are available for generating random numbers from common univariate distributions, and these can often be extended to a multivariate context. For example, random proportions from the Dirichlet distribution can be obtained from independent random gamma variates.

The theory presented here unifies the KF and EV paradigms, both of which have been applied in existing fisheries literature. Although the KF paradigm is attractive computationally, it requires normal error, and it is only approximate if either function **F** or **G** in (1.1)–(1.2) depends nonlinearly on the state vector **X**. For example, the KF paradigm is inapplicable when age proportions are modeled with the multinomial or Dirichlet distributions. Model design involves choices: linear or nonlinear system equations, normal error or more complex stochastic structure. Often the choice requires a balance between mathematical simplicity and complexities inherent in fishery data. The theory here provides a consistent framework within which to develop and compare a spectrum of choices.

The KF paradigm typically involves a fixed set of parameters, regardless of the number $N$ of time periods, whereas the number of parameters increases with $N$ in the EV paradigm. This distinction, however, can become blurred. For example, Sullivan (1992) adopts the KF approach to a catch-at-length model in which a recruitment parameter is introduced for each year $t$. His model does not include a state equation comparable with (CA.7), and recruitments are unrestricted. Thus, Sullivan's model illustrates that the number of parameters can increase with $N$ even in a KF context.

The EV paradigm shares several important features with the analysis of functional relationships (Kendall and Stuart 1979, sect. 29.14). The number of parameters increases with $N$. Knowledge of measurement and process error variance ratios is essential for estimation. Furthermore, in the context of a linear functional relationship, Schnute et al. (1990) show that the negative log likelihood, rather than *twice* the negative log likelihood, should be used for conventional likelihood ratio tests. Such documented problems with functional relationships may similarly apply to the EV paradigm.

Recent developments in computing technology address two important issues in the implementation of state space models. First, new computer languages, such as C++ and FORTRAN 90, offer constructs that make it easier to express such procedures as the KF algorithm in Tables 4 and 5. For example, FORTRAN 90 includes matrix arithmetic intrinsically; operator overloading in C++ makes it possible to define an intrinsic matrix arithmetic; and C++ class structure allows entire algorithms, such as Kalman recursions, to be defined abstractly. Second, automatic differentiation (Griewank and Corliss 1991; Otter Research Ltd. 1991) offers an efficient method for computing the gradients of **F** and **G** in Tables 5 and 6, as well as the likelihood gradient required for maximizing the likelihood function. State space models can involve hundreds of parameters. For example, an EV implementation of Tables 7 and 8 includes $11 + AN$ parameters, the $11 + A$ components of $\mathbf{\Theta}$ in (5.11), and an additional $(A - 1)N$ states. Thus, with 10 age classes and 20 yr of data, the model involves 211 parameters. Within current fisheries literature, this would probably be considered a large number, although modern computing algorithms make it routinely possible to deal with such problems.

At the end of Section 4, I discussed analyses by Little and Rubin (1987) and Mendelssohn (1988) that suggest potential problems with the EV paradigm in which missing data are treated as parameters. Gelb (1974, p. 210) documents similar bias problems with an extended Kalmar filter. The purpose of my paper is not to advocate either the EV or KF paradigm, but rather to place them within the common theoretical framework developed in Section 2 and to show how this framework influences the design of fishery models. In the nonlinear context, both paradigms seek to circumvent computational difficulties associated with high dimensional integration, as in (2.9) and (2.12). Bayes analysts have an obvious interest in such computational problems, and methods are being devised for addressing them (Tierney and Kadane 1986; Smith and Gelfand 1992; Kramer 1993). Thus, although the methods discussed here may be suboptimal in some circumstances, the theoretical development at least equips the reader to enter the debate seriously and to examine new paradigms as they become available. Because no universal theory exists for optimal nonlinear estimation, each model and estimation scheme can be rigorously assessed only via cycles of simulation and estimation.

In my view, the most difficult problem posed by state space models deals with identifiability. For example, the model in Tables 7 and 8 includes seven variance parameters: three correlations and four standard errors. The outcome of the analysis often depends critically on values of these parameters, and it is generally impossible to estimate all of them. For example, if the measurement error $\tau_1$ associated with the index $I_t$ is large enough, then (1) the index bears no relationship to the underlying biomass, (2) the index parameter $q$ cannot be estimated, (3) no absolute measure can be assigned to the states $K_{at}$, and consequently, (4) the population is not identifiable. Statistically, the likelihood surface is "flat", i.e., insensitive to large parameter changes. In cases like this, a large number of conflicting scenarios appear equally consistent with the known data, and the analyst has no objective means to choose among them.

This apparent limitation can be turned into an advantage. State space model design forces essential questions to be asked about underlying processes, observed data, and sources of variability. When these questions are answered honestly, the model may point to scenarios consistent with the data but in conflict with the prevailing view. If so, the modeling effort can help to delineate the limits of current knowledge and to establish rational priorities for future data collection.

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## Appendix A. Proof of (KL.5)–(KL.6)

Harvey (1989, p. 165) proves that if **X** and **Y** are jointly multivariate normal with

$$(A.1)\qquad \begin{pmatrix}\mathbf{X}\\ \mathbf{Y}\end{pmatrix} \sim N\left[\begin{pmatrix}\boldsymbol{\mu}_x\\ \boldsymbol{\mu}_y\end{pmatrix}, \begin{pmatrix}\boldsymbol{\Sigma}_{xx} & \boldsymbol{\Sigma}_{xy}\\ \boldsymbol{\Sigma}_{yx} & \boldsymbol{\Sigma}_{yy}\end{pmatrix}\right],$$

then

$$(A.2)\qquad \mathbf{X}|\mathbf{Y} \sim N[\boldsymbol{\mu}_x + \boldsymbol{\Sigma}_{xy}\boldsymbol{\Sigma}_{yy}^{-1}(\mathbf{Y} - \boldsymbol{\mu}_y), \boldsymbol{\Sigma}_{xx} - \boldsymbol{\Sigma}_{xy}\boldsymbol{\Sigma}_{yy}^{-1}\boldsymbol{\Sigma}_{yx}].$$

In the present context, let $\mathbf{Y}^{t-1}$ be given, and let **X** and **Y** in (A.1) correspond to $\mathbf{X}_t$ and $\mathbf{Y}_t$, respectively. Then

$$(A.3)\qquad \boldsymbol{\mu}_x = \mathbf{M}_{Xt}, \boldsymbol{\Sigma}_{xx} = \mathbf{W}_{Xt}$$

$$(A.4)\qquad \boldsymbol{\mu}_y = \mathbf{M}_{Yt}, \boldsymbol{\Sigma}_{yy} = \mathbf{W}_{Yt}.$$

Furthermore, because $\mathbf{Y}_t = \mathbf{C}_t + \mathbf{D}_t\mathbf{X}_t + \boldsymbol{\epsilon}_t$ with $\boldsymbol{\epsilon}_t$ independent of $\mathbf{X}_t$, it follows that the $m \times n$ covariance matrix $V[\mathbf{X}_t,\mathbf{Y}_t]$ is

$$(A.5)\qquad \boldsymbol{\Sigma}_{xy} = \mathbf{W}_{Xt}\mathbf{D}'_t.$$

Substituting (A.3)–(A.5) into the expressions for the mean and covariance matrix of **X**|**Y** in (A.2) gives the formulas (KL.5)–(KL.6) for $\mathbf{M}^*_{Xt}$ and $\mathbf{W}^*_{Xt}$, respectively. Note that $\boldsymbol{\Sigma}_{yx} = \boldsymbol{\Sigma}'_{xy}$ and that $\mathbf{W}_{Xt}$ is symmetric, i.e., $\mathbf{W}_{Xt} = (\mathbf{W}_{Xt})'$.

## Appendix B. EV Recursions

To prove the recursions in Table 6, regard all EV parameters (2.10) as constants, except for $\boldsymbol{\xi} = (\mathbf{X}_2,\ldots,\mathbf{X}_N)$. It follows from (2.1)–(2.2), (2.7)–(2.8), and (2.11) that

$$(B.1)\qquad -2\log P(\boldsymbol{\xi}) = K + \sum_{t=2}^{N}(\boldsymbol{\delta}'_t\mathbf{U}_t^{-1}\boldsymbol{\delta}_t + \boldsymbol{\epsilon}'_t\mathbf{V}_t^{-1}\boldsymbol{\epsilon}_t),$$

where $P(\boldsymbol{\xi})$ is the Bayes posterior and $K$ is a constant independent of $\boldsymbol{\xi}$. From (B.1) and the definitions (2.3)–(2.4), the gradient conditions $\boldsymbol{\nabla}_{\mathbf{X}_t}P(\boldsymbol{\xi}) = 0$ with $t = 2,\ldots,N - 1$ imply that

$$(B.2)\qquad \mathbf{U}_t^{-1}\boldsymbol{\delta}_t - \mathbf{B}'_{t+1}\mathbf{U}_{t+1}^{-1}\boldsymbol{\delta}_{t+1} - \mathbf{D}'_t\mathbf{V}_t^{-1}\boldsymbol{\epsilon}_t = 0,$$

where

$$(B.3)\qquad \mathbf{B}_{t+1} = \boldsymbol{\nabla}_{\mathbf{X}}F(\mathbf{X}_t,\mathbf{Z}_{t+1},\boldsymbol{\Theta})$$

$$(B.4)\qquad \mathbf{D}_t = \boldsymbol{\nabla}_{\mathbf{X}}G(\mathbf{X}_t,\mathbf{Z}_t,\boldsymbol{\Theta}).$$

The $N - 2$ equations (B.2) and the definitions (B.3)–(B.4) imply the recursions in Table 6, where (B.2) is solved for $\boldsymbol{\delta}_{t+1}$ and $t$ is replaced by $t - 1$.

Incidentally, the condition $\nabla_{\mathbf{X}_N} P(\boldsymbol{\xi}) = 0$ implies that

$$\text{(B.5)} \qquad \mathbf{U}_N^{-1}\hat{\boldsymbol{\delta}}_N - \mathbf{D}_N'\mathbf{V}_N^{-1}\boldsymbol{\epsilon}_N = 0,$$

and this is not used in Table 6. Thus, the $N - 2$ equations (B.2) are inadequate to determine all components of $\boldsymbol{\xi}$. As discussed in connection with (4.4)–(4.5), (B.5) is satisfied only when $\boldsymbol{\xi}$ is computed at the estimate $\hat{\mathbf{X}}_2$ that maximizes (4.5).

# Comparing Polynomial and von Bertalanffy Growth Functions for Fitting Tag–Recapture Data

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The properties of polynomial and von Bertalanffy growth functions are compared for analysing data from tag–recapture experiments in which fish are recaptured once. For the quadratic and von Bertalanffy growth functions, explicit formulae are obtained for the expected growth increment in terms of length-at-release, time-at-liberty, and the function parameters. If the least-squares fitting technique is used the von Bertalanffy function fits tag–recapture data with no more bias (probably less) than any other growth function, including polynomial growth functions. A bias-reduction technique for fitting the von Bertalanffy growth function to tag–recapture data is not applicable to other growth functions. We conclude that, apart from the straight line, the von Bertalanffy growth function is the one with the most desirable mathematical and statistical properties for fitting to tag–recapture data. The matter of the function that best characterises the way a specific fish species grows can be adequately addressed only by analyses of multiple measurements of individual fish.

On compare les propriétés des fonctions de croissance polynomiales et de von Bertalanffy pour analyser les données des expériences de marquage–recapture dans lesquelles les poissons sont recapturés une seule fois. Dans le cas des fonctions de croissance quadratiques et de von Bertalanffy, on obtient des formules explicites pour l'augmentation de croissance prévue en termes de longueur au moment de la remise en liberté, le moment de la remise en liberté et les paramètres de la fonction. Lorsqu'on utilise la technique d'ajustement par la méthode des moindres carrés, la fonction de von Bertalanffy s'ajuste aux données de marquage–recapture en ne produisant pas davantage de biais (en en produisant probablement moins) que toute autre fonction de croissance, y compris les fonctions de croissance polynomiales. Une technique de réduction des biais pour ajuster la fonction de croissance de von Bertalanffy aux données de marquage–recapture ne peut pas s'appliquer à d'autres fonctions de croissance. Nous concluons que, à l'exception de la ligne droite, la fonction de croissance de von Bertalanffy est celle dont les propriétés mathématiques et statistiques sont les plus valables pour s'ajuster aux données de marquage–recapture. Seule l'analyse de plusieurs mesures chez des individus différents permet de déterminer adéquatement quelle fonction est la meilleure pour déterminer la façon dont une espèce particulière de poisson se développe.

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## Polynomial Growth Functions

Recently some researchers have preferred polynomial growth functions (PGF) over the conventional von Bertalanffy (1938) growth function (VBGF)

$$(1)\quad L(t) = L_\infty\{1 - e^{-k(t-t_0)}\}$$

to describe the growth of fish (e.g., Geoghegan and Chittenden 1982; Standard and Chittenden 1984; Brown 1988; Chen et al. 1988). In (1), $L(t)$ is the expected length of a fish at age $t$ and $L_\infty$ the asymptotic maximum length as $t \to \infty$. At $t = t_0$ the expected length is zero and the expected growth rate is $kL_\infty$. The PGF, with as many parameters as the VBGF, is the quadratic

$$(2)\quad L(t) = a + bt + ct^2.$$

The main reasons for preferring PGF over VBGF are that (a) PGF can model a decrease in average length as fish become older (which could happen if, for example, fast-growing individuals had a higher mortality rate than slow-growing ones), (b) PGF may simply fit a given data set better than VBGF, and (c) the limitations of PGF are more obvious than those of VBGF, which might discourage extrapolation of the function to ages not covered by the observed data.

Rafail (1972) provided evidence that PGF fitted empirical length-at-age data from five fish species as well as, or better than, VBGF. Chen et al. (1992), however, found that VBGFs fitted better than PGFs (both quadratic and cubic) to data from 16 populations comprising six fish species. Roff (1980) pointed out that "even if the fish grew according to the von Bertalanffy function the fit obtained with the parabola would be, in most cases, indistinguishable from that obtained with the von Bertalanffy equation." Knight (1968) strongly criticised the extrapolation of VBGF to very old fish, in which $L_\infty$ is interpreted as the maximum length attained, on average, in the population.

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## Tag–Recapture Growth Models

We consider growth models that analyse information from single-recapture tagging experiments (i.e., tagged fish that are recaptured once) and examine the properties (including bias) of analyses based on PGF and VBGF. Tag–recapture data give a type of information about fish growth that is different from length-at-age data. This type is especially common with species of fish that are difficult or impossible to age directly. Alternatively, one may simply have obtained the two kinds of growth data and wish to assess their consistency, as in Kirkwood (1993) and Francis (1988a).

Consider a fish of length $l_1$ that is tagged at age $t_1$ (which is unknown) and recaptured after a known time-at-liberty $\Delta t$ $(= t_2 - t_1)$ later, at age $t_2$ with length $l_2$. The conventional way to analyse these data with the VBGF is to transform (1) to

$$(3) \quad \Delta L = (1 - e^{-k\Delta t})(L_\infty - L_1)$$

where $\Delta L = L_2 - L_1$ and $L_i = E(l_i)$. The expected lengths $L_1$ and $L_2$ are estimated by the observed lengths $l_1$ and $l_2$ (giving observed increment $\Delta l$). The parameters $L_\infty$ and $k$ are estimated by a statistical procedure such as the least-squares method of Fabens (1965), which assumes the variance around $\Delta l$ to be constant. Modifications of Fabens's (1965) basic method were applied by Kirkwood and Somers (1984), Francis (1988b), Maller and deBoer (1988), Hampton (1991), and James (1991), all of which allow the variance around $\Delta l$ to vary.

Estimates derived from fitting (3) can be heavily biased. According to Maller and deBoer (1988) and James (1991), the fundamental problem in Fabens (1965) is that one of the estimating equations is biased: the expected values $L_1$ and $L_2$ are estimated from the measured lengths $l_1$ and $l_2$, and the "residual"

$$(4) \quad \eta = l_2 - l_1 - (L_\infty - l_1)(1 - e^{-k\Delta t})$$

has zero expectation, but an estimating equation uses

$$\eta\Delta t(L_\infty - l_1)e^{-k\Delta t}.$$

The latter expression has nonzero expectation because it is a nonlinear function of $l_1$ which is a random variable, not a parameter. The above-mentioned modifications to Fabens's (1965) method, except for James's (1991), include more complex error structures but retain this bias. James (1991) suggested a solution: multiply $\eta$ by a function of time-at-liberty and the model's parameters instead of a function of the data.

Hearn (1986) briefly discussed some implications of fitting the quadratic PGF to tag–recapture data. He reparameterized (2) to obtain

$$(5) \quad L(t) = L_m\left\{\kappa(t-\tau_0) - \frac{1}{4}\kappa^2(t-\tau_0)^2\right\}$$

so that the parameters are analogous to those of VBGF: $L_m$ is the maximum expected length, at age $t_m = 2/\kappa + \tau_0$, and at $t = \tau_0$ the expected length is zero and the expected growth rate is $\kappa L_m$. The inverse function of (5) is

$$(6) \quad t - \tau_0 = \frac{2}{\kappa}\left\{1 - \sqrt{1 - \frac{L(t)}{L_m}}\right\}.$$

From (5) and (6) the expected growth increment is

$$(7) \quad \Delta L = L_m\left\{\kappa\Delta t\sqrt{1 - \frac{L_1}{L_m}} - \frac{1}{4}(\kappa\Delta t)^2\right\}$$

where, in order to obtain a unique solution, it is assumed that fish are tagged at an age younger than $t_m$, which limits the type of experiments that can be conducted. Then, $\Delta L$ is a decreasing function of age for recapture ages greater than $t_m$, which preserves the ability of PGF to model a decrease in average length-at-age.

As for VBGF, the expected lengths can be estimated by the observed lengths, and the parameters $L_m$ and $\kappa$ can be estimated by least-squares from (7). Accordingly, $L_m$ cannot be smaller than any observed lengths at release, but if this condition is not quite met, (7) may be adjusted by substituting a value of zero in the expression under the square root.

## Comparison of Statistical Properties

The analogue of the residual in (4) for the quadratic function is

$$(8) \quad \eta = l_2 - l_1 - L_m\left\{\kappa\Delta t\sqrt{1 - \frac{l_1}{L_m}} - \frac{1}{4}(\kappa\Delta t)^2\right\},$$

which is already a nonlinear function of $l_1$. Both the estimating equations for $L_m$ and $\kappa$ are therefore biased. A suitable means of correcting these biases could not be found using the approaches applied to VBGF in James (1991, sections 2.2 and 2.3) because the expectations of the estimating functions depend in a complicated way on the unknown expected length $L_1$.

We therefore expect the statistical properties of the estimation of a quadratic growth function to be as poor as, and probably worse than, those of Fabens's (1965) method, and almost certainly worse than those of James's (1991, section 2.3) method.

Consider a general growth function

$$L(t) = f(t - t_0; \theta)$$

where $\theta$ is a vector of parameters that needs to be estimated. The formula for the expected growth increment, analogous to (3), is

$$(9) \quad \Delta L = f(f^{-1}(L_1) + \Delta t) - L_1.$$

For PGFs of degree more than 2 and most other functions, the expression for $f^{-1}(L_1)$ can be defined only implicitly and may have multiple solutions, in which case an appropriate numerical solution is needed in each iteration of the algorithm for fitting data. On the other hand, for the Richards' (1959) growth curve, which generalises the VBGF, logistic, Gompertz, and monomolecular growth functions, an explicit formula for $\Delta L$ can readily be obtained from (9).

James's (1991, section 2.3) method for correcting biased estimating equations requires that the right-hand side of (9) be linear in $L_1$; that is

$$f(f^{-1}(L_1) + \Delta t) = A(\Delta t)L_1 + B(\Delta t).$$

Letting $\Delta t \to 0$ gives

$$\frac{dL}{dt} = \frac{dA}{dt}(0)\,L + \frac{dB}{dt}(0),$$

the solution of which is a VBGF.

All functions, other than the straight line and VBGF, therefore have nonlinear expressions of $\Delta L$ in terms of $L_1$. We expect their parameter estimates to be in general more biased than the VBGF parameters estimated by the James technique.

## Concluding Remarks

If a PGF fits a tag–recapture data set better than VBGF, it "does not necessarily indicate that the von Bertalanffy model is inadequate for the species under consideration" (Sainsbury 1980, p. 246). In such cases the fitted PGF may be applied with caution in some situations. The matter of the function that best characterises the way a fish species grows can be adequately addressed only by separate analyses of many length measurements on individual fish. For instance, Phillips et al. (1983) analysed data from individual western rock lobsters (*Panulirus cygnus*) that had been kept in an aquarium for up to 12 yr. Using the approach of Sandland and McGilchrist (1979), they developed a stochastic differential equation model that fitted the data of individuals better than VBGF.

However, for analysing data from single-recapture tagging experiments, we have demonstrated that VBGF has advantages over PGF and other functions (except the straight line): (a) the mathematics is simpler and (b) the estimating equations for the parameters have less bias. For some practical applications of growth, bias may be of little importance. However, because of the absence of potential problems due to bias, we advise fitting the VBGF to tag–recapture data and using the James (1991, section 2.3) method.

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# Impact of Nutrients and Physical Factors on the Shift from Cyanobacterial to Chlorophyte Dominance in Shallow Danish Lakes

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Jensen, J.P., E. Jeppesen, K. Olrik, and P. Kristensen. 1994. Impact of nutrients and physical factors on the shift from cyanobacterial to chlorophyte dominance in shallow Danish lakes. Can. J. Fish. Aquat. Sci. 51: 1692–1699.

Phytoplankton dominance (as biomass) by heterocystous cyanobacteria, nonheterocystous cyanobacteria, and chlorophytes was studied along a trophic gradient (0.011–2.2 mg P·L$^{-1}$) by analyzing regularly collected semiquantitative data from 178 shallow Danish lakes (mean depth <3 m) and quantitative data from 32 lakes. Heterocystous cyanobacteria were dominant at low total P (TP) (<0.25 mg P·L$^{-1}$) and nonheterocystous cyanobacteria at intermediate TP (0.25–0.8 mg P·L$^{-1}$), while chlorophytes often were dominant at high TP (>1 mg P·L$^{-1}$). In contrast with many earlier findings, heterocystous cyanobacteria were not dominant at low total N (TN):TP or low inorganic N concentrations; chlorophytes were dominant at extremely high pH, and the shift from cyanobacterial to chlorophyte dominance could not be explained by a change in the photic zone to mixing zone ratio. We suggest that chlorophyte dominance in hypertrophic shallow lakes is attributable to continuous input of nutrients and carbon from the sediment and external sources. This renders the fast-growing chlorophytes a superior competitor compared with the relatively slow-growing cyanobacteria, even when inorganic nutrient concentration is low and pH high. New predictive models relating phytoplankton dominance to TP in shallow lakes were developed, as former models failed to predict our observations satisfactorily.

On a étudié les espèces dominantes de phytoplancton (en termes de biomasse), soit les cyanobactéries hétérocystiques, les cyanobactéries non hétérocystiques et les chlorophycophytes le long d'un gradient trophique (0,011–2,2 mg P·L$^{-1}$) en analysant des données semi quantitatives recueillies régulièrement dans 178 lacs danois peu profonds (profondeur moyenne <3 m) et des données quantitatives provenant de 32 lacs. Les cyanobactéries hétérocystiques étaient dominantes à une faible concentration de P total (PT) (<0,25 mg P·L$^{-1}$) et les cyanobactéries non hétérocystiques aux concentrations intermédiaires de PT (0,25–0,8 mg P·L$^{-1}$), et les chlorophytes dominaient souvent à des concentrations élevées de PT (>1 mg P·L$^{-1}$). Contrairement à de nombreux résultats obtenus auparavant, les cyanobactéries hétérocystiques n'étaient pas dominantes à un faible rapport N total (NT):PT ou à de faibles concentrations de N inorganique; les chlorophytes étaient dominants à des pH extrêmement élevés, et le changement de dominance des cyanobactéries au profit des chlorophytes ne pouvait s'expliquer par une modification du ratio zone euphotique à zone de mélange. Nous sommes d'avis que la dominance des chlorophytes dans les lacs hypertrophiques peu profonds est attribuable à un apport continu de nutriments et de carbone provenant des sédiments et de sources externes. Les chlorophytes qui croissent plus rapidement sont donc des concurrents plus sérieux que les cyanobactéries dont la croissance est relativement lente, même à une faible concentration de nutriments inorganiques et à un pH élevé. De nouveaux modèles prévisionnels mettant en relation les espèces dominantes de phytoplancton et la concentration de PT dans les lacs peu profonds ont été élaborés, car les anciens modèles ne permettaient pas de prévoir nos observations de façon satisfaisante.

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Major changes in phytoplankton community structure are known to take place along the trophic gradient, and large cyanobacteria have been found to dominate at high nutrient levels in several lakes. This is also reflected in the predictive models by Trimbee and Prepas (1987) and Seip (1988) who found that cyanobacterial dominance increased with increasing total P (TP). Various explanations of cyanobacterial dominance at high nutrient levels have been proposed, e.g., changes in the availability of P and the relative availability of P and N (total N (TN):TP ratio, light conditions, pH/CO$_2$ conditions, and zooplankton grazing pressure) (e.g., Smith 1986; Pick and Lean 1987; Shapiro 1990). The relative importance of these factors is, however, still subject to considerable debate (Shapiro 1990; Smith 1990).

Although the majority of studies suggest that cyanobacteria are the dominant phytoplankton in eutrophic lakes, there are a few reports on exclusive dominance by chlorophytes, particularly in shallow hypertrophic lakes and ponds (e.g., Nygaard 1949; Pavoni 1963; Leah et al. 1980; Bucka 1989; Jeppesen et al. 1990b, 1990c, 1991), even under conditions that, according to Shapiro (1990) and Smith (1990), should favour cyanobacterial growth (high pH, low TN:TP, CO$_2$, and light). Analyses of data from a number of shallow Danish lakes have revealed that the dominance of chloro-

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TABLE 1. Some characteristics of the lakes studied ($n = 178$). Morphometric data and hydraulic retention time were calculated on a yearly basis, while the remaining parameters were calculated for the summer (May 1 – Oct. 1).

| | Mean | Median | Min. | Max. |
|---|---|---|---|---|
| Surface area ($km^2$) | 0.85 | 0.25 | 0.001 | 41.0 |
| Mean depth (m) | 1.5 | 1.4 | 0.5 | 3.0 |
| Maximum depth (m) | 2.4 | 2.0 | 0.6 | 10.1 |
| Hydraulic retention time (yr) | 0.46 | 0.25 | 0.006 | 4.3 |
| TP (mg $P \cdot L^{-1}$) | 0.52 | 0.38 | 0.011 | 2.2 |
| TN (mg N $L^{-1}$) | 2.8 | 2.2 | 0.20 | 10.9 |
| Secchi depth (m) | 0.70 | 0.55 | 0.20 | 3.0 |
| pH | 8.2 | 8.1 | 6.3 | 10.1 |

phytes at high TP levels was the rule rather than the exception (Jeppesen et al. 1990a, 1991). Jeppesen et al. (1990b) argued that the chlorophyte dominance in hypertrophic Lake Søbygård may reflect the circumstance that phytoplankton, besides using incoming nutrients, also use nutrients and $CO_2$ released at high rates from the lake sediment during summer, a source which is not important for phytoplankton growth in the epilimnion in stratified lakes.

It is commonly believed that especially heterocystous cyanobacteria are particularly abundant in eutrophic lakes with a low content of inorganic N or low N:P in the external loading (Schindler 1977; Smith 1983). However, in shallow Danish lakes, they (typically *Anabaena* and *Aphanizomenon*) dominate in early summer when nitrate concentration is high and the concentration of inorganic P is low in contrast with late summer when nonheterocystous cyanobacteria (typically *Microcystis*) often are dominant (J.P. Jensen, unpublished data). *Microcystis* dominance at low inorganic N concentrations has also been observed elsewhere (Gelin 1975; Gelin and Ripl 1978; Vincent 1989; Takamura et al. 1992). It therefore appears that it is not the low concentration of inorganic N that determines the shift from dominance by nonheterocystous cyanobacteria or chlorophytes to heterocystous cyanobacteria.

In the present paper, we further evaluate the shift from cyanobacterial to chlorophyte dominance and the shift from dominance by heterocystous to dominance by nonheterocystous species of cyanobacteria in shallow lakes. Using semiquantitative data from 178 Danish lakes and quantitative data from 32 lakes, we seek to answer the following questions: Do cyanobacteria or chlorophytes dominate at high nutrient levels, high pH, and low light conditions? Are heterocystous cyanobacteria more dominant at low inorganic N concentrations and low TN:TP ratios than nonheterocystous cyanobacteria and chlorophytes or are other environmental factors more important for the differences in dominance structure? And finally, are the regression models relating cyanobacterial dominance to TP valid for temperate shallow lakes? If not, can we develop new predictive models for this type of lake?

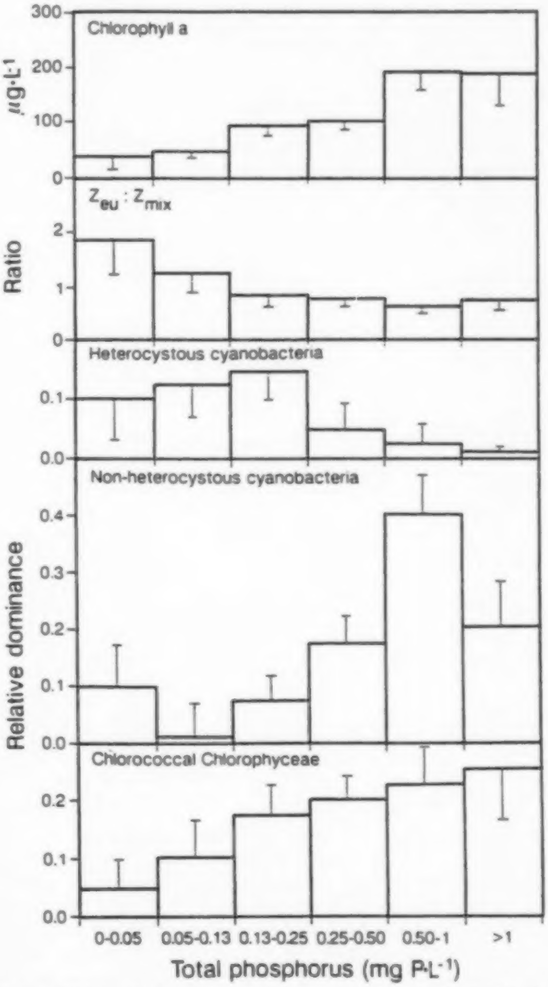

FIG. 1. Chlorophyll *a*, $Z_{mix}$:$Z_{eu}$, and relative cyanobacterial and chlorophyte dominance (±heterocysts) in the six groups of lakes as classified by P (summer mean values, May 1 – Oct. 1). Vertical bars indicate SE of the mean in each group.

## Materials and Methods

### Study Area

The main characteristics of the 178 lakes included in the study are given in Table 1. The lakes were mainly small (mean surface area 0.85 $km^2$) and shallow (mean depth 1.5 m) with a low hydraulic retention time (0.46 yr). Most of the lakes had high external nutrient loading, and although nutrient levels varied widely (0.011–2.2 mg $P \cdot L^{-1}$ and

TABLE 2. Correlations between cyanobacterial (±heterocysts) and chlorophyte dominance and selected environmental parameters (summer mean values, May 1 – Oct. 1) for the semiquantitative data set. The upper value represents Spearman's rank correlation coefficient ($r_s$) (Sokal and Rohlf 1981) and the parenthetical value the corresponding significance level ($P(r_s = 0)$) ($n = 178$).

| | TP | TN | Secchi depth | pH | $Z_{mean}$ | $Z_{eu}:Z_{mix}$ |
|---|---|---|---|---|---|---|
| Cyanobacteria | 0.15 | −0.04 | −0.21 | 0.05 | 0.18 | −0.32 |
| (nonheterocysteous) | (0.05) | (0.62) | (0.01) | (0.08) | (0.03) | (0.00) |
| Cyanobacteria | −0.15 | −0.02 | −0.17 | 0.00 | −0.05 | −0.16 |
| (heterocysteous) | (0.04) | (0.86) | (0.03) | (0.96) | (0.84) | (0.06) |
| Chlorophyceae | 0.19 | 0.06 | −0.07 | 0.25 | 0.02 | −0.06 |
| (chlorococcales) | (0.01) | (0.42) | (0.39) | (0.00) | (0.86) | (0.50) |

0.20–10.9 mg N·L$^{-1}$) the mean concentration of P and N was high (0.52 mg P·L$^{-1}$ and 2.8 mg N L$^{-1}$). Because of the high nutrient levels, chlorophyll *a* tended to be high and exceeded 200 µg·L$^{-1}$ when the TP concentration was above 0.5 mg P·L$^{-1}$ (Fig. 1). Mean Secchi depth was low (0.70 m). The euphotic zone to mixing zone ratio $Z_{eu}:Z_{mix}$ decreased with increasing TP concentration (Fig. 1), but was relatively constant at concentrations above 0.125 mg P·L$^{-1}$.

### Sampling and Analyses

Composite epilimnion sampling was undertaken by local water authorities. The samples were analyzed for various physical and chemical parameters and for phytoplankton composition. TP was measured as orthophosphate using the method of Murphy and Riley (1972) following persulphate digestion (Koroleff 1970). Ammonium was determined by the phenate method (APHA 1985). Nitrite + nitrate was determined as nitrate using a flow injection analyzer, and inorganic N was calculated as the sum of ammonium, nitrite, and nitrate. TN was measured as nitrate + nitrite after potassium persulphate digestion according to the methods of Solórzano and Sharp (1980). The pH of the samples was measured with a pH meter either in the field or in the laboratory. Temperature was measured in the field with either a thermometer or a thermistor with a precision greater than 0.5°C; when temperature gradients were present in the water column, the measurements were made at minimum intervals of 1 m. The euphotic zone was calculated from the Secchi depth ($Z_{eu}$ = 1.7·Secchi depth; Reynolds 1984). The mixing zone was defined as the water column mean depth when there was no temperature gradient and as the zone from the surface to the depth where the temperature gradient exceeded 1°C·m$^{-1}$ in stratified lakes (Ruttner 1963).

Phytoplankton were enumerated on Lugol-fixed sedimented water samples using an inverted microscope (Útermöhl 1958; Rott 1981). Phytoplankton abundance and "biomass" were rated using six categories: absent, present in low numbers, moderately high density and "biomass", high density and "biomass", subdominant in terms of "biomass", and dominant in terms of "biomass" (Olrik 1981). In a few cases (<15%), only four categories were used: absent, present, subdominant in terms of "biomass", and dominant in terms of "biomass". "Biomass" is put in quotation marks because its determination is based on the importance of each individual group as judged from the projected picture in the inverted microscope and not from actual biomass determination. This implies that the importance of, for instance, large spherical species is somewhat underestimated. To ensure that the present analysis represents biomass dominance, only the category "dominant" is used. Systematic classification of phytoplankton was undertaken according to the method of Christensen (1966, 1980), the main identification references being Hüber-Pestalozzi (1933–83), Komárek (1958), and Bourrelly (1966, 1968, 1970). In the present study, we only include chlorococcal green algae in the term chlorophytes, as this group characterizes eutrophic waters (Reynolds 1984), while other greens, e.g., desmids, are found in a larger nutrient spectrum, the latter being particularly abundant under oligotrophic conditions (Nygaard 1949).

For each environmental parameter analyzed, data were arranged in numerical order and then divided into six groups of equal size (approximately 30 lakes in each group). The relative dominance of the three phytoplankton groups was calculated as the proportion of samples in each class of the environmental parameter analyzed in which a given phytoplankton group was dominant in terms of biomass (summer (May–October) means a minimum of four samples). This nonlinear method of data presentation was necessary because of the use of a rating scale to assess the response variable. Basic statistical testing when undertaken according to the method of Sokal and Rohlf (1981) using the SAS statistical package (SAS Institute Inc. 1989), the exception being phytoplankton "biomass" rating versus environmental parameters which was performed by means of logistic regression, this being a more appropriate and powerful statistical method than ordinary linear regression for testing rating scale responses (Hosmer and Lemeshow 1989). Different junctions of the explanatory parameters, e.g., logarithmic, exponential, and polynomial equations, were tested in the logistic procedure. The fitting of the logistic equations was undertaken according to the "logistic procedure" of the SAS/STAT statistical package (SAS Institute Inc. 1989).

In the verification data set from 32 lakes the phytoplankton species composition was determined by counting and the specific volume calculated by fitting the different species and genera to geometric forms (Útermöhl 1958; Edler 1979; Rott 1981). The chemical and physical parameters were determined as described above.

## Results

Correlation analysis on semiquantitative data revealed that dominance by heterocystous cyanobacteria, nonheterocystous cyanobacteria, and chlorophytes was significantly correlated to TP concentration (Table 2). However, whereas dominance by nonheterocystous cyanobacteria (mainly *Microcystis*) and chlorophytes (mainly *Scenedesmus* and *Pediastrum*) increased significantly with increasing TP ($P < 0.05$

TABLE 3. Correlations between cyanobacterial (±heterocysts) and chlorophyte dominance and selected environmental parameters (summer mean values, May 1 – Oct. 1) for the quantitative data set. The upper value represents Spearman's rank correlation coefficient ($r_s$) (Sokal and Rohlf 1981) and the parenthetical value the corresponding significance level ($P(r_s = 0)$) ($n = 32$).

| | TP | TN | Secchi depth | pH | $Z_{mean}$ | $Z_{eu}:Z_{mix}$ |
|---|---|---|---|---|---|---|
| Cyanobacteria | 0.18 | 0.11 | −0.35 | 0.35 | 0.24 | −0.46 |
| (nonheterocysteous) | (0.32) | (0.54) | (0.05) | (0.06) | (0.17) | (0.00) |
| Cyanobacteria | −0.29 | −0.04 | 0.12 | −0.10 | −0.03 | −0.10 |
| (heterocysteous) | (0.10) | (0.84) | (0.53) | (0.59) | (0.86) | (0.59) |
| Chlorophyceae | 0.88 | 0.75 | −0.72 | 0.65 | 0.08 | −0.73 |
| (chlorococcales) | (0.00) | (0.00) | (0.00) | (0.00) | (0.65) | (0.00) |

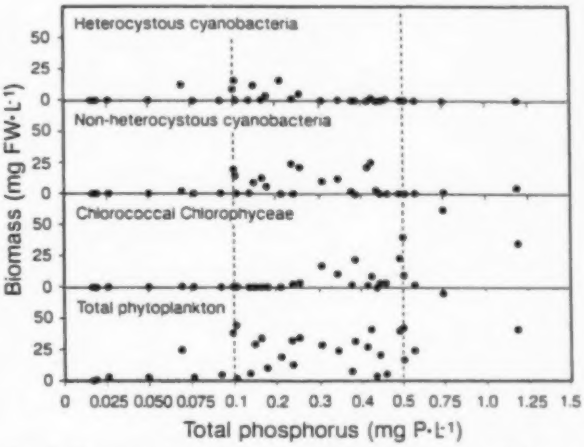

FIG. 2. Biomass of cyanobacteria, chlorophytes, and total phytoplankton in relation to summer TP concentration (mean values, May 1 – Oct. 1).

and $P < 0.01$, respectively), dominance by heterocystous cyanobacteria (mainly *Anabaena* and *Aphanizomenon*) decreased ($P < 0.05$). In contrast, there was no significant correlation between dominance by the three phytoplankton groups and the concentration of TN. In each case, there was a negative correlation with Secchi depth, although this was only significant for the cyanobacteria (both heterocysteous and nonheterocysteus). Dominance by chlorophytes increased significantly with increasing pH whereas the relationship between pH and cyanobacteria was less clear. Even though the range of mean depth was rather narrow (<3 m), a positive correlation could be demonstrated between dominance by nonheterocystous cyanobacteria and mean depth. When $Z_{eu}:Z_{mix}$ increased, dominance by nonheterocystous and heterocystous cyanobacteria decreased, there being no significant correlation in the case of chlorophytes (Table 2). The correlation matrix was similar for the less extensive quantitative data set (Table 3), suggesting that the consistency conclusions for the semiquantitative data below apply to the quantitative data as well.

More detailed analysis of the semiquantitative and quantitative data revealed nonlinear relationships (Fig. 1 and 2). Below 0.5 mg P·L$^{-1}$, nonheterocystous cyanobacteria became increasingly dominant with increasing TP whereas above 1 mg P·L$^{-1}$, dominance was reduced significantly ($P < 0.0041$: Mann–Whitney *U*-test) (Sokal and Rohlf 1981). The relationship between the probability of dominance and TP for the semiquantitative data set was unimodal with a maximum at 0.82 mg P·L$^{-1}$ ($P < 0.0063$), as described by the logistic regression equation

$$(1)\quad P(\text{dominance}) = 1/(1 + \exp(1.9 - 4.6 \cdot \text{TP} + 2.8 \cdot \text{TP}^2)).$$

Dominance by nonheterocystous cyanobacteria was also significantly inversely related to $Z_{eu}:Z_{mix}$ ($P < 0.0089$):

$$(2)\quad P(\text{dominance}) = 1/(1 + \exp(1.7 + 1.6 \cdot \log(Z_{eu}:Z_{mix}))).$$

Heterocystous cyanobacteria tended to dominate at relatively low TP with a maximum between 0.06 and 0.25 mg P·L$^{-1}$ (Fig. 1 and 2). However, an exponentially declining relationship to TP gave the best regression ($P < 0.0063$):

$$(3)\quad P(\text{dominance}) = 1/(1 + \exp(1.2 + 1.4 \cdot \text{TP})).$$

The relationship between dominance by heterocystous cyanobacteria and $Z_{eu}:Z_{mix}$ was only tenuous ($P < 0.0514$), but as with the nonheterocystous cyanobacteria, dominance decreased when the ratio increased:

$$(4)\quad P(\text{dominance}) = 1/(1 + \exp(0.7 + 1.4 \cdot (Z_{eu}:Z_{mix}))).$$

Dominance by chlorophytes increased with increasing TP, in particular above 1 mg P·L$^{-1}$ (Fig. 1 and 2). The best fit was obtained using a square root (sqrt) function ($P < 0.0098$):

$$(5)\quad P(\text{dominance}) = 1/(1 + \exp(1.5 - 1.4 \cdot \text{sqrt}(\text{TP}))).$$

In contrast with the cyanobacteria, there was no significant relationship between dominance by chlorophytes and $Z_{eu}:Z_{mix}$ ($P > 0.3035$). All three groups of phytoplankton were generally more dominant at high pH values (Fig. 3) although dominance by cyanobacteria tended to decrease at the highest pH (pH > 9). The TP–phytoplankton models developed in the present study (equations 1, 3, and 5) deviated markedly from the predictions of Trimbee and Prepas (1987) and Seip (1988) (Fig. 4); cyanobacterial dominance was somewhat lower in our models, and there was no decrease in relative cyanobacterial dominance at high TP. However, the models of Trimbee and Prepas (1987) and Seip (1988) only cover TP up to 0.2 and 1 mg P·L$^{-1}$, respectively.

As with the correlation with TN (Table 3), the relationship between dominance and TN:TP was less clear (Fig. 5). The nonheterocystous cyanobacteria were most frequently dominant at TN:TP less than 8, although no significant relationship could be established ($P > 0.072$). In contrast with Smith (1983), dominance by heterocystous cyanobacteria gradually increased up to a TN:TP of about 32. They were only occasionally dominant above this level. Cumulative distribution of dominance in relation to inorganic N (Fig. 6) suggests

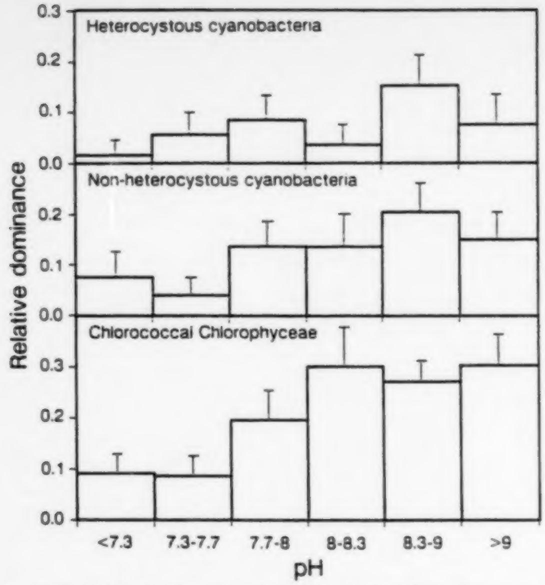

FIG. 3. Relative dominance of cyanobacteria (±heterocysts) and chlorophytes in relation to pH (summer mean values, May 1 – Oct. 1). Vertical bars indicate SE of the mean in each group.

that nonheterocystous cyanobacteria (as well as chlorophytes) were more frequently dominant at low inorganic N concentrations than were heterocystous cyanobacteria. Chlorophytes were only occasionally dominant at TN:TP above 32 (Fig. 5), although in this case, no significant trend could be detected at ratios below this threshold ($P > 0.15$). However, as the median TP and TN values in the six TN:TP groups were 1.2, 0.6, 0.4, 0.2, 0.1, and 0.03 mg $P{\cdot}L^{-1}$ and 1.8, 1.6, 2.4, 1.6, 2.9, and 2.2 mg $N{\cdot}L^{-1}$, respectively, changes in TN:TP, and hence the relationship between TN:TP and phytoplankton, may instead reflect changes in TP.

## Discussion

The general finding of the present study that cyanobacteria become increasingly dominant towards eutrophic conditions is in accordance with numerous other studies (Olrik 1978; Reynolds 1984; Smith 1986; Pick and Lean 1987; Reynolds 1987; Smith et al. 1987; Trimbee and Prepas 1987; Sas et al. 1989). However, there have been few other reports of a shift to dominance by chlorophytes at hypertrophic conditions (Nygaard 1949; Pavoni 1963; Leah et al. 1980; Bucka 1989), probably because most comparative studies have dealt with lakes that are deeper or have less extreme nutrient concentrations, conditions which as discussed below are less favourable for chlorophyte dominance. Thus, whereas chlorophytes are characterized by high growth and loss rates and hence have high demand for nutrients (C-strategist, sensu Reynolds 1988), cyanobacteria have lower growth and loss rates and hence a lower demand for nutrients (S-strategist, sensu Reynolds 1988). The balance between the rates of net growth and loss may be one of the keys to understanding why chlorophytes are usually able to outcompete cyanobacteria at high TP in shallow, nonstratified lakes, but not in deep, stratified lakes. In eutrophic–hypertrophic lakes, nutrient turnover as a result of zooplankton grazing is usually low because of high fish predation (E. Jeppesen et al., personal observation). This means that the turnover of nutrients in the water column is low. The nutrient balance in such lakes is consequently very sensitive to loss by sedimentation unless the nutrients are returned to the photic zone with little or no delay. The latter is the case in nonstratified lakes where sediment nutrient release continuously recycles nutrients lost through sedimentation back to the photic zone. For example, maximum release rates as high as 240 mg $P{\cdot}m^{-2}{\cdot}d^{-1}$ and summer averages of 100 mg $P{\cdot}m^{-2}{\cdot}d^{-1}$ have been observed in hypertrophic chlorophyte-dominated Lake Søbygaard (Søndergaard et al. 1990; Jensen and Andersen 1992) in which daily sedimentation loss comprises 20% of the biomass and 40% of the primary production (Kristensen and Jensen 1987; Jeppesen et al. 1990c). P release from the sediment was more than 10 times the external loading despite a short hydraulic retention time (15–25 d) (Jensen and Andersen 1992). This example, together with the findings from other shallow Danish lakes (Jensen and Andersen 1992), underlines the importance of the sediment as a major source of nutrients for phytoplankton growth in shallow lakes. In addition, the high sediment release rates may help to explain why many hypertrophic lakes are dominated by chlorophytes, even when the concentration of inorganic nutrients is low. In stratified lakes, in contrast, nutrients lost by sedimentation are trapped in the hypolimnion, thereby discouraging growth of algae with high loss rates; nevertheless, chlorophytes may still become dominant during periods of high grazing pressure and hence high nutrient turnover in the photic zone (Sommer 1985; Vanni and Temte 1990).

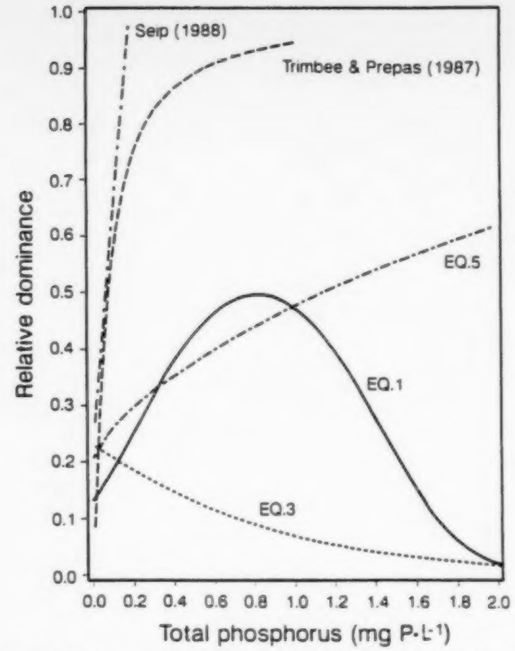

FIG. 4. Comparison of different models relating the relative dominance of phytoplankton to TP. EQ.1, EQ.3, and EQ.5 are the equations for nonheterocystous cyanobacteria, heterocystous cyanobacteria, and chlorophytes, respectively. The equations of Trimbee and Prepas (1987) and Seip (1988) are also shown.

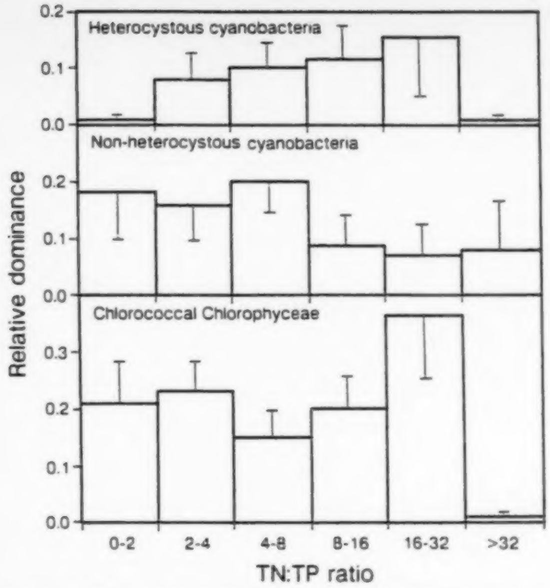

FIG. 5. Relative dominance of cyanobacteria (±heterocysts) and chlorophytes in relation to TN:TP (summer mean values, May 1 – Oct. 1). Vertical bars indicate SE of the mean in each group.

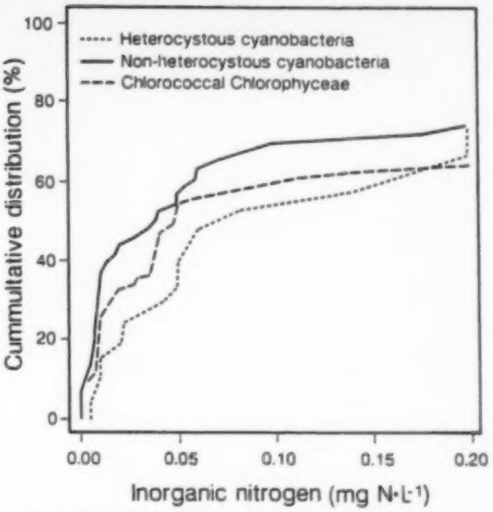

FIG. 6. Cumulative distribution of cyanobacterial (±heterocysts) and chlorophyte dominance in relation to summer inorganic N concentration (May 1 – Oct. 1) up to 0.2 mg N·L$^{-1}$.

Apart from nutrients, the outcome of the competition between cyanobacteria and chlorophytes may be influenced by $Z_{eu}$:$Z_{mix}$, cyanobacteria generally being better adapted to low light conditions than chlorophytes (Zewenboom and Mur 1980). Although this may explain the inverse relationship between dominance and $Z_{eu}$:$Z_{mix}$ seen with both groups of cyanobacteria, it cannot explain the major shift to chlorophytes in hypertrophic lakes (>0.8 mg P·L$^{-1}$), since the ratio was just as low as in the cyanobacteria-dominated lakes.

Physical stability of the water in the photic zone may also influence phytoplankton dominance. Thus, as in the deep stratified lakes, stable water column conditions favour growth of cyanobacteria in eutrophic lakes (e.g., Reynolds and Walsby 1975; Steinberg and Hartmann 1988). Hence, the frequent physical disturbance of the photic zone seen in shallow lakes may disfavour cyanobacteria and may, by causing the pulsed release of nutrients from sediment (e.g., by resuspension), enhance the competitiveness of chlorophytes, as has been demonstrated in the laboratory (Sommer 1985).

Variations in zooplankton grazing pressure have been shown to affect the relative phytoplankton composition in many lakes, and large phytoplankton species are often favoured in lakes with high grazing pressure (e.g., Bergquist et al. 1985). It may, therefore, be argued that the low zooplankton grazing pressure in hypertrophic lakes as a result of high fish predation may be a prerequisite for the dominance of small-sized edible chlorophytes instead of large inedible cyanobacteria. In contradiction to this view, however, results from a number of Danish hypertrophic lakes have shown that increasing grazing pressure on phytoplankton due to a reduction in fish predation on zooplankton leads to a shift from dominance by small-sized to dominance by large-sized chlorophytes (typically from *Scenedesmus* or *Chlorella* to *Pediastrum* or *Oocystis*, etc.) (Jeppesen et al. 1990b; E. Jeppesen et al., personal observation). Only at almost fish-free conditions was a shift to dominance by grazerblade forms of *Aphanizomenon* found, as also observed in other studies (Fott et al. 1980; Lynch 1980; Andersson and Cronberg 1984).

Several authors have stressed the fact that cyanobacteria are favoured at high pH/low $CO_2$ because of their lower half-saturation constant for $CO_2$ uptake (King 1970; Talling 1976; Shapiro 1984). Shapiro (1990) even claimed that "if the pH/$CO_2$ hypothesis is correct it alone could go a long way towards explaining blue-green dominance in lakes, and changes in that dominance". In contrast, DeNoyelles and O'Brien (1978) found that chlorophytes dominated at pH values above 10.5 in enriched experimental ponds and they were apparently able to take up sufficient C for growth under these conditions. These results are in accordance with the findings of the present study, since chlorophyte dominance was more positively correlated with pH than was cyanobacterial dominance (Fig. 3). Furthermore, exclusive dominance by chlorophytes has even been observed at pH values as high as 11.2 (Jeppesen et al. 1990a). These contradictory findings may be attributable to a difference in the input of atmospheric $CO_2$, the input in shallow lakes being greater per unit of volume than in deeper lakes because of the higher surface area to volume ratio (Olrik 1981). In addition, and probably more importantly, shallow nonstratified lakes are continuously supplied with $CO_2$ from the sediment.

The ability of heterocystous cyanobacteria to fix atmospheric N should be advantageous when the ambient inorganic N concentration is low (Paerl 1988) or when TN:TP is low (Smith 1983). In the present study, cyanobacteria were seldom dominant at TN:TP above 32, a level close to the threshold of 29 proposed by Smith (1983) for differentiating cyanobacteria-dominated lakes (Fig. 5). However, the negative relationship between increasing TN:TP and cyanobacterial dominance proposed by Smith (1985) was not found and, in fact, heterocystous cyanobacterial dominance increased with TN:TP up to a ratio of 32. Moreover, heterocystous cyanobacteria were not the superior competitors at low inorganic N concentrations (Fig. 6); they mainly occurred in early summer, when inorganic N concentration was relatively high and ortho-P low as compared with later in the summer (J.P. Jensen, unpublished data). Our findings are more in accordance with the conclusions of Trimbee and Prepas (1987) who, by recompiling the data of Smith (1985), concluded that the shift to cyanobacterial dominance at low TN:TP was caused by the increase in TP rather than the decrease in TN:TP. Whether the energetically rather costly $N_2$-fixation is important at low inorganic N concentrations in shallow nonstratified lakes is therefore open to question. Leonardson (1984), who studied $N_2$-fixation of heterocystous cyanobacteria in two shallow eutrophic lakes, concluded that $N_2$-fixation is generally a minor N contributor to phytoplankton growth in such lakes. In analogy with P release, the sediment N flux may be an important N source in shallow lakes (Søndergaard et al. 1990), thereby rendering shallow lakes less favourable to atmospheric $N_2$-fixation than deeper lakes. In addition, whereas shallow eutrophic lakes are often highly supersaturated with $O_2$ throughout the water column, this probably inhibiting the strictly anaerobic $N_2$-fixation process, cyanobacteria in deep lakes may reach areas of low $O_2$ during the night, e.g., in the metalimnion. Our data strongly suggest that the outcome of competition between heterocystous and nonheterocystous cyanobacteria in the lakes of the present study is determined by higher P affinity of the former rather than by variation in the ability to fixate atmospheric N.

In some aspects, our findings were discordant with those of Trimbee and Prepas (1987), and also of Seip (1988), whose models predicted a higher relative cyanobacterial dominance in eutrophic lakes than did our models and no decrease in cyanobacterial dominance at high TP (Fig. 4). This discrepancy might be attributable to differences in the lake types studied, the analysis of Trimbee and Prepas (1987) covering lakes with a mean depth ranging from 1.1 to 313 m, the majority being deep (mean 27 m), while that of Seip (1988) mainly included deep lakes, the precise depths of which were not given. Accordingly, their model predictions fit well with observations from other deep lakes (Sas et al. 1989; Faafeng et al. 1990), TP threshold for cyanobacterial dominance being about 10 $\mu g\ P \cdot L^{-1}$, there being no upper limit for cyanobacteria dominance, and no shift to chlorophytes at high TP such as found in the shallow lakes of the present study.

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# Isolation, Partial Characterization, Induction, and the Occurrence in Plasma of the Major Vitelline Envelope Proteins in the Atlantic Halibut (*Hippoglossus hippoglossus*) during Sexual Maturation

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Hyllner, S.J., B. Norberg, and C. Haux. 1994. Isolation, partial characterization, induction, and the occurrence in plasma of the major vitelline envelope proteins in the Atlantic halibut (*Hippoglossus hippoglossus*) during sexual maturation. Can. J. Fish. Aquat. Sci. 51: 1700–1707.

The vitelline envelope of Atlantic halibut (*Hippoglossus hippoglossus*) is composed mainly of two major and two minor proteins. Estradiol-17ß induces the two major vitelline envelope proteins in halibut of both sexes. These proteins were also found in plasma of vitellogenic females. The origin of the two major vitelline envelope proteins is not restricted to the ovary, as male halibut synthesize these proteins after treatment with estradiol-17ß. Individual female halibut were followed and plasma sampled from May to March. Plasma levels of estradiol-17ß increased in October, peaked in early February, and declined until the last ovulation in March. In October, the two major vitelline envelope proteins were first detected in plasma and the amount remained elevated until early March. Prior to the last ovulation, the amount decreased. Plasma vitellogenin varied in a similar way. The results provide strong evidence that the physiological regulation of the synthesis of the major vitelline envelope proteins in teleosts is controlled by the female sex steroid estradiol-17ß.

L'enveloppe vitelline du flétan atlantique (*Hippoglossus hippoglossus*) est principalement composée de deux protéines majeures et de deux protéines mineures. L'oestradiol-17ß induit la production des deux protéines majeures de l'enveloppe vitelline chez les flétans des deux sexes. Ces protéines se retrouvent également dans le plasma de femelles en vitellogenèse. L'origine des deux protéines majeures de l'enveloppe vitelline n'est pas limitée à l'ovaire, car le flétan mâle synthétise ces protéines après un traitement à l'oestradiol-17ß. On a suivi des flétans femelles et prélevé chez elles des échantillons de plasma de mai à mars. Les concentrations d'oestradiol-17ß dans le plasma ont augmenté en octobre, ont atteint leur maximum au début de février et ont diminué par la suite jusqu'à la dernière ovulation en mars. En octobre, les deux protéines majeures de l'enveloppe vitelline ont d'abord été décelées dans le plasma et leur concentration est demeurée élevée jusqu'au début de mars. Avant la dernière ovulation, la concentration a diminué. La vitellogénine plasmatique variait de la même façon. Ces résultats indiquent clairement que la régulation physiologique de la synthèse des protéines majeures de l'enveloppe vitelline chez les téléostéens est effectuée par l'oestradiol-17ß, une hormone stéroïdienne sexuelle femelle.

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During oocyte development in vertebrates, an acellular envelope is formed outside the oolemma (Dumont and Brummet 1985). The terminology and biological functions of the egg envelope vary in different vertebrate groups. In teleosts, the envelope is often referred to as the vitelline envelope and forms a tough protective coat around the egg and embryo. Recent research has provided insight into the composition, regulation, and origin of the vitelline envelope in teleost fish.

The composition of the vitelline envelope has been investigated in six teleost species; it consists of two to four major proteins (Hamazaki et al. 1987; Begovac and Wallace 1989; Oppen-Berntsen et al. 1990; Brivio et al. 1991; Hyllner et al. 1991; Iuchi et al. 1991; Masuda et al. 1991). In rainbow trout (*Oncorhynchus mykiss*), the vitelline envelope is composed of three major proteins that are related but distinct (Hyllner et al. 1991), and it has been suggested that the teleost vitelline envelope proteins represent a separate class of structural proteins (Hagenmaier 1985; Hyllner et al. 1991).

An endocrine regulation of the major protein constituents of the egg envelope has been demonstrated in teleost fish.

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Estradiol-17ß induces the major vitelline envelope proteins in rainbow trout, brown trout (*Salmo trutta*), turbot (*Scophthalmus maximus*) (Hyllner et al. 1991), Japanese medaka (*Oryzias latipes*) (Murata et al. 1991), and Atlantic cod (*Gadus morhua*) (Oppen-Berntsen et al. 1992b). Estradiol-17ß is an important female sex steroid hormone, which also regulates the synthesis of vitellogenin, the yolk protein precursor. Vitellogenin is synthesized in the liver and transported in the blood to the oocytes, where it is incorporated, transformed into yolk proteins, and stored, i.e., vitellogenesis (Wallace 1985). During vitellogenesis, the amount of the major vitelline envelope proteins in rainbow trout plasma was found to increase concomitantly with plasma estradiol-17ß (Hyllner and Haux 1992). The major vitelline envelope proteins have also been detected in the blood of sexually mature female Japanese medaka (Murata et al. 1991).

So far, the changes of the major vitelline envelope proteins in plasma during female sexual maturation have only been described for rainbow trout (Hyllner and Haux 1992). The rainbow trout is a salmonid fish that can spawn a single batch of eggs each year. The growth of the oocytes is reflected by increases in plasma estradiol-17ß and vitellogenin, as well as in the major vitelline envelope proteins (Scott and Sumpter 1983; Copeland et al. 1986; Hyllner and Haux 1992). The Atlantic halibut (*Hippoglossus hippoglossus*) is a periodic spawner, releasing multiple batches of eggs at regular intervals during a period of 3–6 wk (Norberg et al. 1991). The increases in plasma levels of estradiol-17ß and vitellogenin in halibut are similar to the increases in rainbow trout until the first ovulation (Hyllner and Haux 1992; Methven et al. 1992). However, during the spawning period, multiple peaks of both estradiol-17ß and vitellogenin occur and appear to be related to the ovulatory cycle of the individual fish. These fluctuations have been suggested to reflect vitellogenic cycles in groups of oocytes, while other oocytes are recruited into maturation (Methven et al. 1992).

Both sexes have the potential to synthesize the major vitelline envelope proteins after treatment with estradiol-17ß, which indicates that the site of synthesis is outside the ovary (Hyllner et al. 1991; Murata et al. 1991; Oppen-Berntsen et al. 1992b). Furthermore, it was recently reported that hepatocytes, prepared from rainbow trout treated with estradiol-17ß in vivo, produce vitelline envelope proteins in vitro (Oppen-Berntsen et al. 1992a).

The primary aim of the present study was to investigate the protein composition of the vitelline envelope in the Atlantic halibut. A specific homologous antiserum was developed and used to demonstrate the appearance of vitelline envelope proteins in juvenile halibut treated with estradiol-17ß as well as in blood from maturing females. The intention was to follow the changes of vitellogenin, estradiol-17ß, and the major vitelline envelope proteins in plasma of individual females during sexual maturation.

## Materials and Methods

### Fish and Holding Conditions

Mature halibut were captured by local fishermen and brought to the broodstock facilities of the Austevoll Aquaculture Research Station in southwestern Norway (60°N). The fish were kept in covered, outdoor tanks with a volume of 80 $m^3$. The tanks were supplied with seawater from 50 m depth, with an average salinity of 33–35‰. The water temperature fluctuated between 5 and 12°C throughout the year and was 5–6°C during the spawning season from February to April and reached 8–12°C in August and September. The fish were fed ad libitum three times a week on a diet of whole Atlantic herring (*Clupea harengus*), supplemented with a vitamin mix (H. Rabben, personal communication). Before the start of the study in May 1989, the fish had been kept 4 yr in captivity. The size of the seven females that were sampled ranged from 130 to 150 cm with a weight range of 30–60 kg. The size of the male fish sampled ranged from 70 to 90 cm with a weight range of 5–10 kg.

Juvenile halibut were raised at the rearing facilities at the Austevoll Aquaculture Research Station and kept in indoor round tanks with a diameter of 3 m and a volume of 15 $m^3$. The tanks were supplied with filtered seawater from 50 m depth, with the same salinity and temperature fluctuation as for adult fish. The fish were fed ad libitum daily with moist pellets (Austevoll Fiskeindustri A/S, Norway). The fish used in the present study were 2-yr-olds with a weight range from 0.5 to 1 kg.

### Hormone Treatment and Sampling of Juvenile Fish

Juvenile halibut of both sexes were transferred to 1 × 1 m square tanks with a volume of 0.3 $m^3$ and a water temperature of 6–8°C. After acclimation for 1 wk, the fish were given intraperitoneal injections of 5 mg estradiol-17ß (Sigma, St. Louis, Mo.)·$kg^{-1}$·$wk^{-1}$ for 3 wk. The total dose was 20 mg estradiol-17ß·$kg^{-1}$. Control fish were not injected.

Before sampling, juvenile fish were anaesthetized in 5 mg metomidate (1-(1-phenylethyl)-1H-imidazole-5-carboxylic acid methyl ester·$mL^{-1}$) (Norsk Medisinaldepot, Norway). Blood was collected from the caudal vessels into a precooled, heparinized syringe. The blood samples were immediately centrifuged at 4°C and the resulting plasma was frozen on dry ice and stored at −80°C.

To investigate the effect of proteolytic inhibitors on the stability of vitelline envelope proteins and vitellogenin, a second estradiol-17ß induction experiment was performed. Six treated juveniles were injected intraperitoneally with 100 μL of aprotinin (Sigma), corresponding to a total of 120 trypsin inhibitor units, 30 min prior to sampling. This treatment is effective in preventing proteolysis of vitellogenin in other teleost species (Silversand et al. 1993). Plasma samples from each fish were divided into 100-μL portions. All samples were directly frozen on dry ice. One of the samples was thawed and frozen five times before analysis. After initial freezing, a second and third sample from the same individual were kept at 4 and 20°C, respectively, for 48 h before analysis in order to investigate the sensitivity of the vitelline envelope proteins and vitellogenin to freezing and thawing.

### Sampling of Blood and Eggs of Sexually Maturing Female Halibut

During ovarian recrudescence and growth, blood samples were taken every second month. During the spawning season, blood samples were taken at 7- to 14-d intervals and the maturational stage of the female checked (Norberg et al. 1991). Samples were collected from the duct of Cuvier into a precooled, heparinized syringe and transferred to Eppendorf tubes kept on ice. After centrifugation for 5 min at 2000*g* and 4°C, plasma was frozen on dry ice and stored at −80°C until analyzed. The fish were not anaesthetized during blood collection.

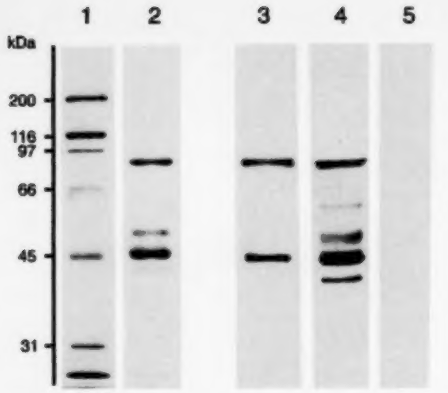

FIG. 1. Lanes 1 and 2: Coomassie Blue stained SDS–PAGE using discontinuous polyacrylamide gels with a 4% stacking gel and a 9% separating gel. (1) Broad-range molecular weight SDS–PAGE standard proteins; (2) approximately 20 μg of purified and solubilized vitelline envelope proteins from Atlantic halibut. Lanes 3–5: Western blot staining of purified and solubilized vitelline envelope proteins. (3) 0.2 μg and (4 and 5) 0.5 μg from halibut subjected to SDS–PAGE and transferred to nitrocellulose sheets; (3 and 4) antiserum directed against halibut vitelline envelope proteins diluted 1:8000; (5) antiserum directed against halibut vitellogenin diluted 1:100 000. Molecular masses are indicated on the left margin of the figure.

Mature females were stripped according to their individual ovulatory cycles, as described in Norberg et al. (1991). For the isolation and characterization of vitelline envelope proteins, five portions of 20 mL of freshly ovulated, unfertilized eggs were frozen on dry ice and stored at −80°C until processed. The fertilization rate of the remaining eggs from the same batch was 95%, indicating that the timing of stripping was correct (Norberg et al. 1991).

### Vitelline Envelope Protein Extraction

Frozen eggs were directly immersed in a large volume of an ice-cold solution of 100 mM EDTA and 500 mM NaCl. The eggs were homogenized with a glass-Teflon homogenizer. The vitelline envelopes were pelleted for 5 min at 150*g*. Pellets were rinsed for 24 h at 4°C in five changes of an ice-cold solution of 500 mM NaCl with mild sonication for 10 s during the first two washes and centrifuged after each wash for 5 min at 150*g*. Pellets were then washed for 5 h at 4°C in two changes of deionized water (Millipore, Massachusetts) to remove salts and centrifuged after each wash for 5 min at 150*g*. The separated vitelline envelope fraction was placed on a watchglass, dried at 35°C for 72 h, and stored in sealed tubes at room temperature.

The vitelline envelopes were solubilized, 1 $\mathrm{mg \cdot mL^{-1}}$, in a Tris-buffered extraction buffer (100 mM Tris–HCl, pH 8.8, 8 M urea, 2% (w/v) SDS, 300 mM 2-mercaptoethanol, 10 mM EGTA) for 30 min at 70°C. The sample was sonicated (Branson Sonifier B-12 with an output of 50 W) at room temperature for 30 s. The sample was then heated to 70°C for 5 min before the sonication procedure was repeated.

### Preparation of Antiserum

Antisera against vitelline envelope proteins from halibut were raised in 12-wk-old New Zealand white rabbits given subcutaneous injections with 100–250 μg of protein, emulsified in Freund's complete adjuvant (Sigma). Booster injections of 100–250 μg of protein, emulsified in Freund's incomplete adjuvant, were given 6 wk after the first injection and thereafter every third week. Blood was sampled, 5 $\mathrm{mL \cdot rabbit^{-1}}$, from an ear vein 10 d after each booster injection. The last blood sampling, 2 wk after a booster injection, was performed by bleeding out through arteria femoralis. The blood was allowed to clot at 4°C overnight. The serum was separated from the clot by centrifugation at 5000*g* for 30 min at 4°C and stored in 0.5-mL samples at −80°C. Antiserum directed against halibut vitellogenin was from the same blood sampling that was used for the vitellogenin RIA described by Methven et al. (1992).

### Gel Electrophoresis and Western Blots

Sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to Laemmli (1970) using discontinuous polyacrylamide gels with a 4% (w/v) stacking gel and a 9% (w/v) separating gel (Hyllner et al. 1991). Electrophoresis was run at 25 mA, constant current, per gel. Prior to electrophoresis, the plasma samples were diluted 1:200 and the vitelline envelope preparation 1:4, with sample buffer (Tris–HCl (60 mM), pH 6.8, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (w/v) glycerol, 0.001% (w/v) bromophenol blue), and heated at 95°C for 4 min. Two identical gels were run on every occasion. One gel was used for detection of vitelline envelope proteins and the other gel was used to detect vitellogenin. The gels were run simultaneously to obtain as similar conditions as possible in order to facilitate comparison of results.

Upon completion of electrophoresis, the samples were electroblotted onto nitrocellulose membranes (0.45 μm) as described by Towbin et al. (1979). The procedure was carried out at 4°C for 1 h at 250 mA. Immune detection was performed during agitation at room temperature by initially blocking against nonspecific binding for 1 h with 3% (w/v) gelatin in a Tris-buffered saline (TBS; 25 mM Tris, 500 mM, NaCl pH 7.5). The nitrocellulose membranes were washed three times for 5 min with Tween-20–TBS (TTBS; 0.05% polyoxyethylene sorbitan monolaurate in TBS) before incubation for 18 ± 1 h with the appropriate primary antiserum solution (1:8000 or 1:100 000 dilution in 1% (w/v) gelatin–TTBS). The nitrocellulose membranes were rinsed three times for 5 min in TTBS before incubation for 1 h with the second antiserum solution (horseradish peroxidase – conjugated goat antirabbit polyvalent antibody in 1:3000 dilution with 1% gelatin–TTBS). Nitrocellulose membranes were then rinsed twice for 5 min in TTBS and for 10 min in TBS. Visualization was accomplished by using a freshly prepared colour development solution (0.05% (w/v) Bio-Rad horseradish peroxidase colour development reagent (Bio-Rad, California) (0.015% (v/v) $H_2O_2$, 17% (v/v) methanol in TBS).

### Hormone Analysis

Plasma estradiol-17β levels were measured by radioimmunoassay, as described by Norberg et al. (1989), with the following modification: after extraction of 100 μL of halibut plasma with ether–heptane (4:1) and evaporation of the solvent, 1.0 mL of 50 mM phosphate-buffered saline (pH 7.0) was added and the resulting preparation was heated to 60°C

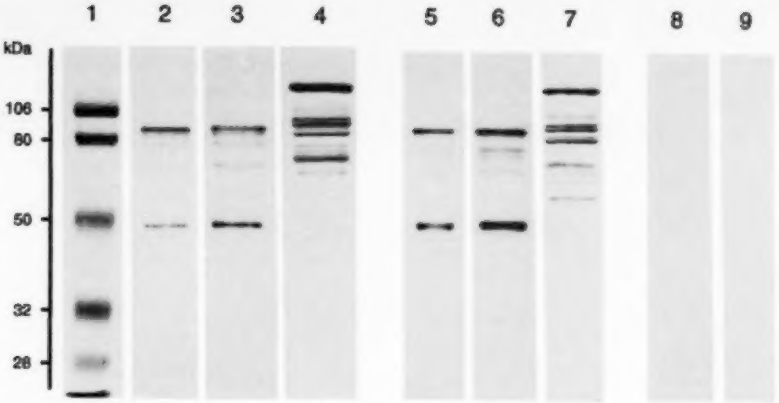

FIG. 2. Western blot staining of SDS–PAGE gels transferred to nitrocellulose. (1) Prestained low molecular weight SDS–PAGE standard proteins. Lanes 2–4: plasma from estradiol-17β-treated juvenile male Atlantic halibut. (2) 20 nL of plasma; (3) 40 nL of plasma; (2 and 3) antiserum directed against halibut vitelline envelope proteins diluted 1:8000; (4) 40 nL of plasma; antiserum directed against halibut vitellogenin diluted 1:100 000. Lanes 5–7: plasma from female halibut sampled 2 mo prior to first ovulation. (5) 20 nL of plasma; (6) 40 nL of plasma; (5 and 6) antiserum directed against halibut vitelline envelope proteins diluted 1:8000; (7) 40 nL of plasma; antiserum directed against halibut vitellogenin diluted 1:100 000. Lanes 8 and 9: plasma from untreated juvenile halibut. (8) 40 nL of plasma; antiserum directed against halibut vitelline envelope proteins diluted 1:8000; (9) 40 nL of plasma; antiserum directed against halibut vitellogenin diluted 1:100 000. Molecular masses are indicated on the left margin of the figure.

for 10 min. This procedure resulted in an average recovery of 89.2 ± 5.2% ($n = 10$) for estradiol-17β from halibut plasma. Anti-estradiol-17β antibody was purchased from ICN Immunobiologicals Inc. (U.K.). Details on the cross-reactivity of the estradiol-17β antibody are given in Methven et al. (1992).

## Results

### Protein and Antiserum Characterization

Two major proteins with molecular masses of 93 and 46 kDa and two minor proteins with molecular masses of 55 and 41 kDa were identified when the solubilized vitelline envelope of halibut was analyzed by SDS–PAGE (lane 2, Fig. 1). The Western blot technique, using homologous antiserum directed against vitellogenin, was used to verify that the isolated and solubilized vitelline envelope preparation was free from vitellogenin or immunoreactive vitellogenin degradation products (lane 5, Fig. 1). The Western blot technique was used to investigate the cross-reactivity between the antiserum directed against homologous vitelline envelope proteins and the vitelline envelope preparation (lanes 3 and 4, Fig. 1). The results revealed that the antiserum primarily cross-reacted with the two major vitelline envelope proteins (lane 3, Fig. 1). However, when loading more protein on the gel, e.g., about 0.5 μg, the antiserum cross-reacted with at least four more proteins (lane 4, Fig. 1), the two minor vitelline envelope proteins with molecular masses of 55 and 41 kDa and two additional proteins with molecular masses between 80 and 60 kDa.

### Occurrence of Vitelline Envelope Proteins in Plasma

Two proteins were immunologically detected in plasma of estradiol-7β-treated juvenile male halibut but not in plasma of control fish using homologous antiserum directed against the solubilized vitelline envelope preparation (lanes 2 and 8, Fig. 2). The two detected estradiol-17β-induced plasma proteins had the same molecular masses as the two major proteins of the vitelline envelope and were not vitellogenin or immunoreactive vitellogenin degradation products (lane 4, Fig. 2). When increasing the amount of plasma loaded to the gel, i.e., from 20 to 40 nL, some additional proteins cross-reacted with the antiserum directed against the vitelline envelope proteins (lane 3, Fig. 2). These proteins were also induced by estradiol-17β, as none of them was detected in the plasma of control fish (lanes 3 and 8, Fig. 2). The induced proteins were not vitellogenin or immunoreactive vitellogenin degradation products because their molecular masses were not matched to any of the proteins that cross-reacted with the antiserum directed against vitellogenin (lanes 3 and 4, Fig. 2). Immunodetection of plasma from vitellogenic females revealed the occurrence of the two major vitelline envelope proteins (lane 5, Fig. 2). Moreover, the additional proteins detected in plasma of hormone-treated fish were also found in vitellogenic females (lane 6, Fig. 2).

### Sensitivity to Degradation of Plasma Vitelline Envelope Proteins and Vitellogenin

When analyzing plasma vitelline envelope proteins and vitellogenin of halibut using the Western blot technique, it was observed that repeated freezing of the plasma samples resulted in an increase in the number of immunoreactive proteins (results not shown). Therefore, an additional experiment was conducted using aprotinin during blood sampling. Vitellogenin was then detected as one single protein band in plasma, thawed once, from halibut treated with estradiol-17β (lane 6, Fig. 3). Repeated freezing and thawing or storage at 4 or 20°C resulted in the appearance of several smaller pro-

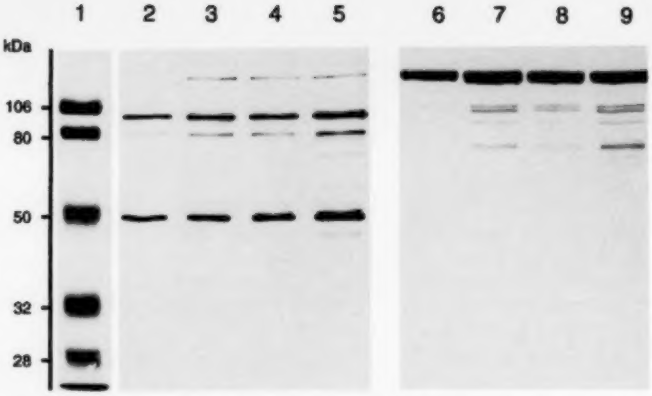

FIG. 3. Western blot staining of SDS–PAGE gels transferred to nitrocellulose. (1) Prestained low molecular weight SDS–PAGE standard proteins. Lanes 2–5: 40 nL of plasma from estradiol-17β-treated juvenile Atlantic halibut; antiserum directed against halibut vitelline envelope proteins diluted 1:8000; (2) plasma thawed once before analysis; (3) plasma thawed five times before analysis; (4) plasma thawed once and stored at 4°C for 48 h before analysis; (5) plasma thawed once and stored at 20°C for 48 h before analysis. Lanes 6–9: 40 nL of plasma from estradiol-17β-treated juvenile halibut; antiserum directed against halibut vitellogenin diluted 1:100 000; (6) plasma thawed once before analysis; (7) plasma thawed five times before analysis; (8) plasma thawed once and stored at 4°C for 48 h before analysis; (9) plasma thawed once and stored at 20°C for 48 h before analysis. Molecular masses are indicated on the left margin of the figure.

teins that cross-reacted with the antiserum directed against vitellogenin (lanes 7–9, Fig. 3). The same pattern was observed for the two major vitelline envelope proteins. In plasma that was thawed once, only the two major vitelline envelope proteins were clearly detected, and storage at 4 or 20°C or repeated freezing resulted in the appearance of several immunoreactive proteins (lanes 3–5, Fig. 3).

### Changes of Estradiol-17β, Vitelline Envelope Proteins, and Vitellogenin in Plasma during Sexual Maturation

The results from the two sampled females presented, halibut 9 and 956, were selected because they were representative of the total group of sampled females, and the collected plasma samples from these two halibut were the most complete. Plasma estradiol-17β in these two females was not detectable in May (below 1.2 ng·mL$^{-1}$). In halibut 9, estradiol-17β increased from 1.6 ng·mL$^{-1}$ in July to 43 ng·mL$^{-1}$ in early February. The first batch of eggs was collected from halibut 9 on 11 February 1990 and the last batch was collected on 27 March 1990. Plasma estradiol-17β decreased after the first ovulation, but remained elevated throughout the ovulatory period. In mid-March, estradiol-17β had decreased to 7 ng·mL$^{-1}$. Halibut 956 followed the same pattern, with an increasing plasma estradiol-17β level until the beginning of February and the first batch of eggs was collected on 4 March 1990. Thereafter, there was a decline in plasma estradiol-17β, and just prior to the last ovulation on March 25, plasma estradiol-17β was not detectable.

The major vitelline envelope proteins were not detected in plasma until October when plasma estradiol-17β increased (Fig. 4). From December to February, the amount of the two major vitelline envelope proteins in plasma remained relatively stable as determined by immunoblotting. Prior to the last ovulation, the amount decreased and some immunoreactive proteins with lower molecular masses appeared.

Besides the two major vitelline envelope proteins, some additional proteins cross-reacted with the antiserum directed against vitelline envelope proteins (Fig. 4). These proteins, detected in most plasma samples, had the same molecular masses as the proteins detected in plasma of hormone treated halibut, as described above. These proteins were not vitellogenin or immunoreactive vitellogenin degradation products because the molecular masses showed no match to any of the proteins that cross-reacted with the antiserum directed against vitellogenin (Fig. 4).

A relatively low amount of vitellogenin was detected in May in plasma of halibut 9. Thereafter, the changes in plasma vitellogenin were similar to the changes of the two major vitelline envelope proteins. When plasma estradiol-17β increased in October, plasma vitellogenin was detected and the amount remained elevated from December to early March in both halibut 9 and 956. In parallel to the changes of vitelline envelope proteins in plasma, the amount of vitellogenin declined before the last ovulation and some additional immunoreactive degradation products of vitellogenin appeared.

## Discussion

The vitelline envelope of Atlantic halibut eggs is mainly composed of four proteins, two major with molecular masses of 93 and 46 kDa and two minor with molecular masses of 55 and 41 kDa. The number of vitelline envelope proteins in halibut is in accordance with observation in other teleost species. Thus, in rainbow trout the vitelline envelope consists of three major proteins (Hyllner et al. 1991), while Brivio et al. (1991) and Iuchi et al. (1991) included a fourth protein as a major constituent. Similarly, the vitelline envelope of Japanese medaka is composed of three (Hamazaki et al. 1987) or four major proteins (Masuda et al. 1991). The Atlantic cod vitelline envelope is composed of three major

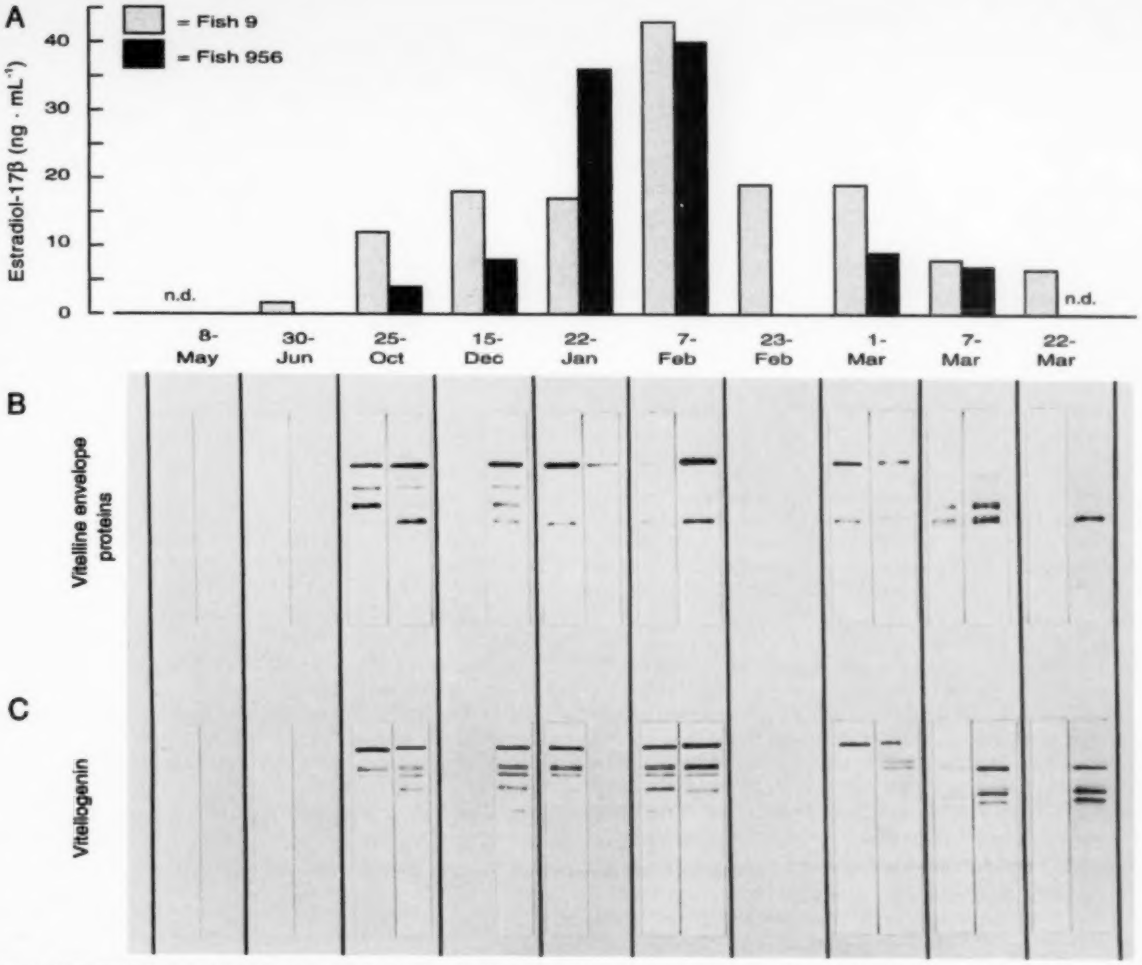

FIG. 4. Seasonal changes in plasma estradiol-17ß, vitelline envelope proteins, and vitellogenin in Atlantic halibut. (A) Seasonal changes in plasma levels of estradiol-17ß in two females (9 and 956); (B and C) Western blot staining of SDS–PAGE gels transferred to nitrocellulose. The gels were loaded with 40 nL of plasma from females 9 (left) and 956 (right). (B) Antiserum directed against halibut vitelline envelope proteins diluted 1:8000; (C) antiserum directed against halibut vitellogenin diluted 1:100 000. n.d. = plasma estradiol-17ß was below 1.2 ng·mL$^{-1}$. Missing data depend on lack of samples.

proteins (Oppen-Berntsen et al. 1990) and the vitelline envelope of pipefish, brown trout, and turbot has two major protein constituents (Begovac and Wallace 1989; Hyllner et al. 1991). The general pattern is that the rather large teleost vitelline envelope is composed of a few major proteins.

The present study demonstrates that estradiol-17ß induces the two major vitelline envelope proteins in juvenile halibut. Such an endocrine control is also present in several other teleost species. More specifically, in rainbow trout, Japanese medaka, and Atlantic cod, the three major vitelline envelope proteins are induced by estradiol-17ß (Hyllner et al. 1991; Murata et al. 1991; Oppen-Berntsen et al. 1992b), and the two major vitelline envelope proteins in brown trout and turbot are also induced by estradiol-17ß (Hyllner et al. 1991). In conclusion, all studies on teleosts have thus far provided clear evidence that estradiol-17ß is the steroid hormone that controls the synthesis of the major vitelline envelope proteins.

In the first induction experiment, several additional protein bands in plasma from halibut treated with estradiol-17ß were observed to cross-react with the antiserum directed against vitelline envelope proteins. These immunoreactive proteins were not found in control fish and were therefore assumed to be vitelline envelope proteins induced by estradiol-17ß. The same plasma samples were found to contain vitellogenin and several fragments of degraded vitellogenin (Fig. 2). The degradation of vitellogenin is a well-known phenomenon in teleost fish and was probably due to the sampling procedure used (Silversand et al. 1993). The suspicion that halibut vitelline envelope proteins may be sensitive to proteolysis required a second induction experiment, where the necessary precautions were taken to prevent degradation. Thus, when the protease inhibitor aprotinin was used during sampling according to the procedures described by Silversand et al. (1993), vitellogenin was detected as one protein band, and the antiserum directed against vitelline envelope proteins cross-reacted primarily with the two major vitelline envelope proteins. Repeated thawing or storage of these plasma samples at room temperature resulted in the appearance of several fragments of degraded vitellogenin as well as several immunoreactive bands considered to be vitelline envelope proteins. On the basis of immunoreactivity, estradiol-17β inducibility, molecular masses, and the results from the aprotinin experiment, the additional immunoreactive proteins observed in the present study are suggested to be degradation products of the vitelline envelope proteins. The sensitivity of teleost vitelline envelope proteins to degradation has not been described earlier.

The two major vitelline envelope proteins were detected in plasma of vitellogenic female halibut. This observation is in agreement with previous findings on sexually mature female Japanese medaka (Murata et al. 1991) and rainbow trout (Hyllner and Haux 1992). The present study is the first to demonstrate the occurrence of the major vitelline envelope proteins in the blood of a marine teleost during sexual maturation. During sampling of maturing females, the sensitivity of plasma vitelline envelope proteins was not realized and aprotinin was therefore not used during sampling. However, the pattern of detected immunoreactive proteins in plasma of maturing females is similar to the proteins observed in plasma from juveniles treated with estradiol-17β, where no aprotinin was used during sampling (Fig. 2). Therefore, we assume that the immunoreactive proteins observed in addition to the two major vitelline envelope proteins are degradation products of vitelline envelope proteins.

The two major vitelline envelope proteins were first detected in plasma in October when plasma estradiol-17β increased. These findings are similar to results obtained in rainbow trout where the amount of plasma vitelline envelope proteins was elevated when plasma estradiol-17β increased (Hyllner and Haux 1992). However, the present study investigated changes in individual females during sexual maturation, which facilitated a direct comparison of the different plasma parameters. Thus, plasma levels of the two major vitelline envelope proteins and vitellogenin were elevated and relatively stable from October until February, while plasma estradiol-17β increased and subsequently decreased. The halibut releases about 10 batches of eggs at regular intervals in the spawning season (Norberg et al. 1991). As blood samples were collected at 7- to 14-d intervals and the fish ovulated at 70- to 80-h intervals, the sampling protocol of the present study did not allow detection of possible fluctuations, related to the ovulatory period. This may account for the relatively stable plasma levels of the two major vitelline envelope proteins and vitellogenin during this period. As one batch of eggs is reaching final maturation, a new batch is recruited into maturation while remaining oocytes may still be vitellogenic. Endocrine regulation of oocyte growth and maturation in periodic spawners is likely to be an intricate interplay between different hormones, but has yet received little attention. For the moment, it can be speculated that when a new batch of eggs is recruited, the two major vitelline envelope proteins and vitellogenin are taken up into the follicle, which would lower the plasma levels. However, as estradiol-17β levels increase, the rate of protein synthesis will increase accordingly, and the plasma levels of the two major vitelline envelope proteins and vitellogenin will therefore remain relatively stable. As the spawning season proceeds, the number of oocytes recruited into maturation and finally ovulated will increase and the number of vitellogenic oocytes decrease. This may explain, at least in part, the gradual decrease in the levels of estradiol-17β during the ovulatory period, as this steroid is produced in the ovarian follicles. The present results on the plasma levels and changes of estradiol-17β and vitellogenin are in general accordance with a previous study on Atlantic halibut (Methven et al. 1992). In addition, the present study investigates and follows the vitelline envelope proteins during sexual maturation. The same pattern regarding the changes in plasma estradiol-17β, vitellogenin, and vitelline envelope proteins before ovulation has previously been observed in a group of female rainbow trout (Hyllner and Haux 1992). Altogether, these findings provide evidence to suggest that estradiol-17β is the steroid hormone that controls the synthesis of the major vitelline envelope proteins during sexual maturation in teleosts.

In the present study, male halibut synthesized vitelline envelope proteins after treatment with estradiol-17β. Since males lack ovaries, some other organ must have the potential to synthesize vitelline envelope proteins, which indicates an extraovarian origin of these proteins. Thus, the present study supports the suggestion that the major vitelline envelope proteins originate from the liver (Hyllner et al. 1991; Murata et al. 1991).

The present paper describes the isolation, hormonal regulation, and plasma changes of the major vitelline envelope proteins in the Atlantic halibut during sexual maturation. In conclusion, the vitelline envelope of halibut is mainly composed of two major and two minor proteins. In both sexes of halibut, estradiol-17β induces the two major vitelline envelope proteins. These two proteins were detected in females during sexual maturation and the plasma levels increased in October and remained elevated until early March. Prior to the last ovulation, the amount decreased. Together, the results strengthen the hypothesis that the biosynthesis of the major vitelline envelope proteins in teleosts is controlled by the female sex steroid estradiol-17β during oocyte development.

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# Influence of Environmental Factors on Heavy Metal Concentration in Lake Sediments in Southern Norway Indicated by Path Analysis

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Fjeld, E., S. Rognerud, and E. Steinnes. 1994. Influence of environmental factors on heavy metal concentration in lake sediments in southern Norway indicated by path analysis. Can. J. Fish. Aquat. Sci. 51: 1708–1720.

We studied relationships between environmental factors and accumulation of Hg, Cd, Pb, and Ni in sediments in 132 lakes in southern Norway. Generally, the concentrations of Hg, Cd, and Pb in surface sediments were considerably elevated as compared with sediments deposited in preindustrial times whereas Ni concentrations showed only minor increases. By path analysis, we modelled how different environmental factors may influence the metal concentrations in surface sediments. The models indicated that both Hg and Pb were associated with sedimenting organic matter, and sediment concentrations were strongly influenced by atmospheric deposition (indirectly measured as metal concentration in mosses) and the amount of organic carrier particles in lake water. Cd behaved similarly, except for a weaker association with organic matter and a positive effect from pH, indicating that Cd is mobilized during acidification. Ni was associated with the inorganic fractions in lake sediments and seemed also to be mobilized during acidification. Geochemical contributions of Ni were larger in sediments than atmospheric deposition whereas atmospheric deposition was the major source of Pb. For the other two elements, estimates of geochemical contributions were lacking.

Nous avons étudié les rapports entre les facteurs environnementaux et l'accumulation de Hg, de Cd, de Pb et de Ni dans les sédiments dans 132 lacs dans le sud de la Norvège. En général, les concentrations de Hg, de Cd et de Pb dans les sédiments de surface étaient beaucoup plus élevées que dans les sédiments déposés à l'époque pré-industrielle, alors que les concentrations de Ni ont peu changé. Dans le cadre d'une analyse causale, nous avons utilisé des modèles pour déterminer comment différents facteurs environnementaux pouvaient influencer la concentration des métaux dans les sédiments de surface. Les modèles ont indiqué que le Hg et le Pb étaient associés à la matière organique en sédimentation et que la concentration dans les sédiments était fortement influencée par les dépôts atmosphériques (indirectement mesurés par la concentration des métaux dans les mousses) et par la quantité de particules organiques agissant comme vecteur dans l'eau des lacs. Le Cd se comportait de la même façon, sauf que l'association avec la matière organique était plus faible et que le pH avait un effet positif, ce qui indique que le Cd est mobilisé durant l'acidification. Le Ni était associé aux fractions inorganiques des sédiments lacustres et semblait également mobilisé durant l'acidification. Le Ni dans les sédiments provenait davantage de sources géochimiques que des dépôts atmosphériques, alors que les dépôts atmosphériques étaient la principale source de Pb. En ce qui concerne les deux autres éléments, on ne disposait pas de mesures sur l'origine géochimique.

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Anthropogenic processes, such as fossil fuel combustion, waste incineration, use of leaded gasoline, and various metallurgical and chemical industries, have caused increased emissions of heavy metals to the atmosphere (Nriagu and Pacyna 1988). In the absence of long-term monitoring of the deposition of heavy metals, lake sediment chemistry has been used to reconstruct the history of atmospheric deposition (Norton et al. 1990). Sediments are especially suitable because metals are adsorbed by fine-grained particles that eventually end up as bottom deposits (Håkanson and Jansson 1983). However, there is not a simple correspondence between atmospheric deposition rates and sediment accumulation rates due to the influence of factors such as sediment focusing (Ohle 1962), bioturbation (Håkanson and Jansson 1983), chemical diagenesis (Carignan and Tessier 1985), acidification (Schindler et al. 1980; Evans et al. 1983), and loading from the catchment area (Dillon et al. 1988; Borg and Johansson 1989). Thus, factors controlling metal concentrations in sediments can be complex, often interrelated, and may influence each other. Special attention has been paid to the contamination of surface sediments because of their potential role in contaminating fish, wildlife, and humans (Burton 1991; Adams et al. 1992). Pb, Cd, Hg, and Ni are among the heavy metals for which anthropogenic

TABLE 1. Summery statistics of variables describing the lakes. All concentrations in sediments are given per dry weight. Lake sediments refer to surfacial sediments (0–1 cm depth). SE = standard error of the mean; $n = 132$.

| Variable | Abbreviation | Unit | Mean | SE | Median | Range |
|---|---|---|---|---|---|---|
| Lake area | $A_l$ | $km^2$ | 8.04 | 1.7 | 0.95 | 0.02–156 |
| Catchment area | $A_c$ | $km^2$ | 317.2 | 73.9 | 20.2 | 0.7–5094 |
| Catchment area ratio | $A_{ratio}$ |  | 36.1 | 4.08 | 18 | 265.7–2.0 |
| Maximum depth | $z$ | m | 40 | 4.6 | 24.5 | 4–306 |
| Altitude | alt. | m | 396 | 27 | 293 | 8–1204 |
| Areal water load | qs | $m \cdot yr^{-1}$ | 32 | 3.7 | 18.5 | 1.8–335 |
| pH in lake water |  |  | 5.7 | 0.7 | 5.8 | 4.3–7.5 |
| Total organic carbon in lake water | TOC | $mg \cdot L^{-1}$ | 3.3 | 0.3 | 2.3 | 0.1–14.8 |
| Loss on ignition | LOI | % | 34.0 | 1.2 | 35.0 | 7.0–68.0 |
| Organic carbon in lake sediments | $OC_s$ | $mg \cdot g^{-1}$ | 15.8 | 0.6 | 15.7 | 0.7–35.5 |
| Hg in lake sediments | $Hg_s$ | $ng \cdot g^{-1}$ | 217 | 11 | 190 | 50–740 |
| Hg in mosses | $Hg_m$ | $ng \cdot g^{-1}$ | 98 | 2 | 100 | 50–160 |
| Pb in lake sediments | $Pb_s$ | $\mu g \cdot g^{-1}$ | 167 | 9 | 151 | 17–567 |
| Pb in mosses | $Pb_m$ | $\mu g \cdot g^{-1}$ | 22 | 1 | 19 | 2–78 |
| Pb in overbank sediments | $Pb_{os}$ | $\mu g \cdot g^{-1}$ | 30 | 2 | 25 | 7–104 |
| Cd in lake sediments | $Cd_s$ | $ng \cdot g^{-1}$ | 1000 | 69 | 780 | 50–3420 |
| Cd in mosses | $Cd_m$ | $ng \cdot g^{-1}$ | 292 | 11 | 270 | 40–790 |
| Ni in lake sediments | $Ni_s$ | $\mu g \cdot g^{-1}$ | 22.4 | 1.3 | 21 | 3–85 |
| Ni in mosses | $Ni_m$ | $\mu g \cdot g^{-1}$ | 2.4 | 0.1 | 2.1 | 0.3–10.8 |
| Ni in overbank sediments | $Ni_{os}$ | $\mu g \cdot g^{-1}$ | 13.4 | 0.7 | 11 | 2–44 |
| Fe in lake sediments | $Fe_s$ | $mg \cdot g^{-1}$ | 93 | 6 | 111 | 5–348 |

atmospheric emissions have resulted in elevated regional depositions and also have caused most ecological problems (Norton et al. 1990).

Our primary objectives in this study were to examine the empirical relationships between Pb, Cd, Hg, and Ni concentrations in surface sediments, atmospheric deposition patterns, and environmental characteristics of lakes in southern Norway and by statistical models test the explanatory power of a number of proposed causal mechanisms. Because of the complex interplay between factors regulating the sediment concentrations, we regarded conventional regression analysis as an inappropriate statistical tool to evaluate the effects of different explanatory variables. In a multiple regression the structure of the model is fixed, allowing only for direct effects between independent and dependent variables, leaving the correlations between the independent variables unanalyzed. We believe that path analysis (Wright 1934) is better suited to study such systems. In a path analysis, one makes explicit assumptions about the nature and directions of the causal relationships, and the causal effects of the explanatory variables are broken down into indirect and direct components. We use this approach in the present study and model how atmospheric deposition of heavy metals may be modified by environmental factors to surface sediment concentrations.

## Materials and Methods

This study is based on observational data from 132 lakes in southern Norway (Fig. 1). These lakes constitute a subsample from a national survey of 210 lakes investigated for heavy metal contamination in sediments (Rognerud and Fjeld 1993). All lakes are dimictic drainage lakes and have no known local sources of heavy metal pollution; they are generally oligotrophic and with low alkalinity and are susceptible to acidification.

We used data for 21 variables for building our path models (see Table 1 for summary statistics and abbreviations of the variables). The variables represent overall morphometric and chemical characteristics of the lakes and their catchment areas,

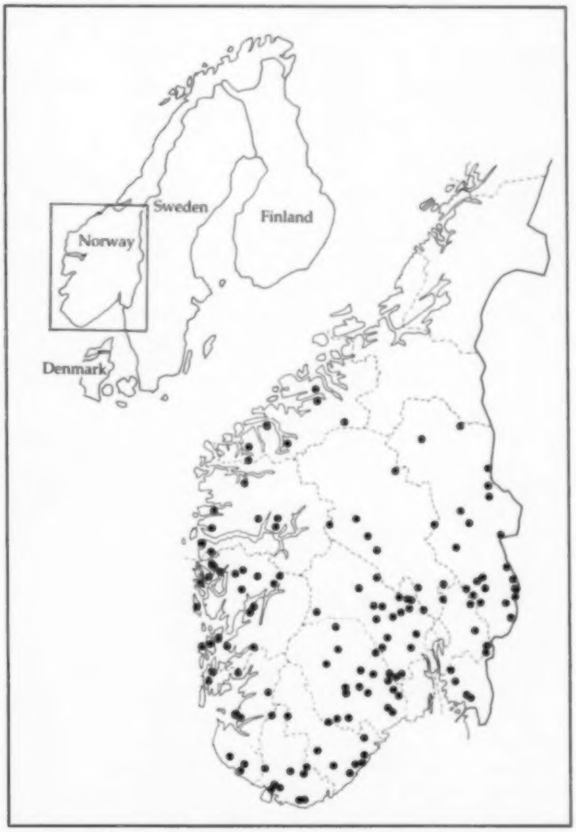


FIG. 1. Location of the study lakes ($n = 132$).

TABLE 2. Correlation matrix for the variables used in the path modelling. Most variables are transformed in

| Variable | Transformed | $A_l$ | $A_c$ | $A_{ratio}$ | $z$ | alt. | qs | pH | TOC | LOI |
|---|---|---|---|---|---|---|---|---|---|---|
| $A_l$ | log | 1.00 | | | | | | | | |
| $A_c$ | log | 0.87 | 1.00 | | | | | | | |
| $A_{ratio}$ | log | 0.14 | 0.61 | 1.00 | | | | | | |
| $z$ | log | 0.69 | 0.59 | 0.08 | 1.00 | | | | | |
| alt. | log | 0.00 | 0.00 | 0.00 | −0.12 | 1.00 | | | | |
| qs | log | 0.05 | 0.42 | 0.78 | 0.19 | −0.02 | 1.00 | | | |
| pH | | 0.40 | 0.47 | 0.29 | 0.18 | −0.02 | 0.08 | 1.00 | | |
| TOC | log | −0.07 | 0.10 | 0.33 | −0.31 | −0.14 | −0.10 | 0.15 | 1.00 | |
| LOI | | −0.52 | −0.57 | −0.30 | −0.28 | −0.06 | −0.26 | −0.50 | 0.23 | 1.00 |
| $OC_s$ | | −0.55 | −0.57 | −0.27 | −0.32 | −0.07 | −0.24 | −0.47 | 0.25 | 0.97 |
| $Hg_s$ | log | −0.05 | −0.15 | −0.22 | 0.24 | −0.26 | −0.15 | −0.41 | −0.02 | 0.58 |
| $Hg_m$ | log | −0.18 | −0.21 | −0.14 | −0.07 | −0.31 | −0.18 | −0.35 | 0.06 | 0.27 |
| $Pb_s$ | log | −0.20 | −0.37 | −0.42 | 0.08 | −0.26 | −0.25 | −0.43 | −0.16 | 0.53 |
| $Pb_m$ | log | −0.19 | −0.22 | −0.13 | 0.01 | −0.42 | −0.12 | −0.44 | −0.02 | 0.26 |
| $Pb_{os}$ | log | −0.15 | −0.18 | −0.12 | 0.11 | −0.28 | 0.06 | −0.35 | −0.25 | 0.14 |
| $Cd_s$ | log | 0.13 | 0.10 | 0.00 | 0.04 | −0.31 | −0.33 | 0.19 | 0.34 | 0.13 |
| $Cd_m$ | log | −0.13 | −0.18 | −0.14 | −0.04 | −0.31 | −0.25 | −0.41 | 0.19 | 0.26 |
| $Ni_s$ | log | 0.30 | 0.27 | 0.06 | 0.21 | −0.27 | 0.06 | 0.41 | −0.17 | −0.49 |
| $Ni_m$ | log | −0.12 | −0.15 | −0.09 | −0.10 | −0.21 | −0.15 | −0.17 | 0.13 | 0.03 |
| $Ni_{os}$ | log | 0.08 | 0.12 | 0.12 | 0.06 | −0.19 | 0.10 | 0.39 | −0.17 | −0.37 |
| $Fe_s$ | log | 0.18 | 0.05 | −0.20 | 0.27 | −0.04 | −0.15 | −0.01 | −0.10 | 0.16 |

and some are either known or suspected to be involved in the processes affecting heavy metal accumulation in sediment.

### Lake Water

Water chemistry data were available from a national acidification survey (Henriksen et al. 1988). The water samples were collected at the outlets of the lakes during the fall (1986) after the lakes had turned over. The climatic conditions (including precipitation) in Norway during 1986 were close to a 30-yr normal. Values for pH (Orion model 801) and TOC (Astro model 2850) from fall overturn were an acceptable estimate for a yearly mean value in 1986 (A. Henriksen, Norwegian Institute of Water Research, personal communication).

We could not estimate theoretical residence times of lake water because we lacked estimates of the lake volumes. This is unfortunate, as we suspect this to be an important factor regarding the retention and sedimentation of heavy metals in lakes. However, we have used the areal water load (qs) as a substitute for residence time. This is defined as the annual water discharge from the lake catchment area divided by the lake area. Because of the correlation between lake depth and volume, qs expresses some of the variation in lake water residence time.

### Lake Sediments

A sediment core was collected with a gravity corer from the deepest part of each lake during the years 1986–88. Each core was split in 1-cm layers and then deep-frozen and sent for analysis. In the path models, we use data from the upper 1-cm surface layer. We also provide data from the deeper part of the core from 85 lakes, usually from a depth between 20 and 30 cm, to document changes in the deposition of heavy metals to the sediments. We define this layer as the "reference layer", likely to have been deposited in preindustrial times and therefore representing the natural background levels of metals. The sedimentation rates in Nordic forest lakes have been studied in several surveys (El-Daoushy and Johansson 1983; Tolonen and Jaakkola 1983; Rekolainen et al. 1986; Renberg 1986), and the mean sedimentation rates for sediment depths below 5 cm varied between 0.7 and 1.7 $mm \cdot yr^{-1}$, while the sediment growth in the upper 5 cm may be somewhat higher due to an elevated water content. From these results, we infer that the surface sediments (0–1 cm) in most of our lakes have mainly been deposited during the 1980's, while reference values correspond to sediment about 200 yr old.

Human influence and natural events in watersheds may cause changes in the basic composition and accumulation rates of sediments, which in turn affect the concentration of heavy metals (Renberg 1986). We therefore inspected every core, looking for changes in color and consistency that could indicate large and abrupt changes in mineral matter accumulation. A small number of lakes with such sediments were excluded.

### Moss Samples

Concentrations of Hg, Pb, Cd, and Ni in the terrestrial feather moss *Hylocomium splendens* were used as a relative measure of the atmospheric deposition of these elements (Rühling and Tyler 1970). Data based on a national sampling program in 1985 (Steinnes et al. 1994), representing the average deposition loads during the period 1982–84, were used. Estimates of relative deposition rates to each of the lakes concerned were based on moss data from representative nearby locations. A description of the sampling procedures used and discussion of the feasibility of the moss techniques in studies of heavy metal deposition are provided by Rühling et al. (1987) and Steinnes et al. (1992).

### Overbank Sediments

We also had access to data on concentrations of Pb and Ni in overbank sediments. These inorganic sediments are deposited on the riverbanks during periods of flooding. The samples are supposed to represent 200- to 1000-yr deposits and are good indicators of the geochemistry of the catchment order to improve normality (log: logarithmic transformed, base 10).

| OC | $Hg_s$ | $Hg_m$ | $Pb_s$ | $Pb_m$ | $Pb_{os}$ | $Cd_s$ | $Cd_m$ | $Ni_s$ | $Ni_m$ | $Ni_{os}$ | $Fe_s$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1.00 | | | | | | | | | | | |
| 0.54 | 1.00 | | | | | | | | | | |
| 0.27 | 0.35 | 1.00 | | | | | | | | | |
| 0.49 | 0.74 | 0.40 | 1.00 | | | | | | | | |
| 0.24 | 0.44 | 0.65 | 0.61 | 1.00 | | | | | | | |
| 0.12 | 0.38 | 0.29 | 0.51 | 0.61 | 1.00 | | | | | | |
| 0.15 | 0.37 | 0.34 | 0.39 | 0.36 | 0.11 | 1.00 | | | | | |
| 0.25 | 0.38 | 0.51 | 0.52 | 0.78 | 0.50 | 0.37 | 1.00 | | | | |
| −0.45 | −0.11 | −0.06 | −0.05 | −0.06 | 0.09 | 0.22 | −0.12 | 1.00 | | | |
| 0.05 | 0.16 | 0.31 | 0.39 | 0.56 | 0.34 | 0.34 | 0.51 | 0.19 | 1.00 | | |
| −0.34 | −0.27 | −0.26 | −0.18 | −0.18 | −0.03 | 0.02 | −0.22 | 0.60 | −0.04 | 1.00 | |
| −0.05 | 0.27 | −0.03 | 0.25 | 0.01 | 0.12 | −0.02 | 0.01 | −0.07 | −0.11 | −0.07 | 1.00 |

areas. A detailed description of the sampling techniques used is provided by Ottesen et al. (1989). The most representative site for each lake was selected from a national survey of overbank sediments. The Norwegian Geological Survey, Trondheim, provided these data. Previously we have shown that the concentrations of Pb and Ni in overbank sediments correlate well with those in unpolluted reference sediments of lakes (Pb: $r = 0.75$, Ni: $r = 0.80$, $n = 150$) (Rognerud and Fjeld 1993). This suggests that overbank sediments may be regarded as good indicators of the geochemical conditioned input of heavy metals to lakes.

The data from the lake water, surface sediments, and moss samples are all comparable in time, and we want to emphasize the importance of having such data as a base for the statistical modelling.

### Analytical Methods

Freeze-dried samples of lake sediment were analyzed for loss on ignition (LOI) and metals after digestion with concentrated $HNO_3$ under pressure (120°C). Concentrations of Pb, Cd, and Ni were determined by graphite furnace atomic absorption spectrophotometry (AAS). Fe was determined by flame AAS. Hg was determined utilizing cold-vapor AAS (Hatch and Ott 1968). Detection limits were as follows: Hg, 0.05 $\mu g \cdot g^{-1}$; Pb, 0.25 $\mu g \cdot g^{-1}$; Cd, 0.05 $\mu g \cdot g^{-1}$; Ni, 2.5 $\mu g \cdot g^{-1}$; Fe, 20 $\mu g \cdot g^{-1}$. Values below detection limits were only found for Hg in reference sediments, and we regard this not to be a major obstacle, as reference sediments are not used in our statistical models. Standard reference sediments (NBS 1645) were submitted randomly among samples before analysis. The analytical results were systematically lower than certified total values. However, the deviation was less than 20% (Hg, 13%; Cd, 15%; Pb, 2%; Ni, 11%). This is because the nitric acid degestion method does not dissolve refractory silicates. The precision varied for different elements. The coefficient of variation was as follows: Hg, 5%; Pb, 6%; Ni, 23%; Cd, 30%. We regard the detection limits and precision generally to be adequate for this study.

Organic carbon in sediments ($OC_s$) may be estimated by LOI and Fe in sediments ($Fe_s$), as organic carbon together with water associated with ferric hydroxide is included in the LOI values (D. Hongve, National Institute of Public Health, Oslo, personal communication):

$$OC_s = 0.52(LOI - 0.48Fe_s),\ R^2 = 0.81,\ n = 241.$$

The moss samples were digested in a solution of concentrated $HNO_3$ and $HClO_4$ (4:1) (Steinnes 1980). Pb was determined by AAS and Ni and Cd by graphite AAS. Determination of Hg was carried out with cold-vapor AAS (Hatch and Ott 1968).

The analysis of overbank sediments was carried out on grain fractions <0.062 mm. This is also the predominant grain size in accumulation sediments of lakes (Håkanson and Jansson 1983). After digestion with concentrated $HNO_3$, concentrations of Pb were determined by AAS whereas Ni concentrations were determined using inductively coupled argon plasma atomic emission spectroscopy (Ødegård 1981). Analysis of duplicate samples, collected and prepared by a different field team, showed a good reproducibility of both Pb and Ni ($r = 0.98$ and 0.95, respectively; $n = 19$).

### Statistical Analysis

Because the distributions of environmental quality data are usually positively skewed, and pollutants sometimes are very highly skewed, we had to transform most of our data to improve normality, stabilize variance, and remove influence of outliers (Table 2).

To compute summary statistics of Hg concentrations in reference sediments, we had to estimate probable values for all data below the detection limit (<50 $ng \cdot g^{-1}$, 30 observations out of 85). We used one of the robust methods recommended by Helsel (1990). We randomly set the censored data to slightly different values below the detection limit and computed normal scores for all observations, including above-limit data. We then made a linear regression of log concentrations on normal scores for all above-limit data. By extrapolating

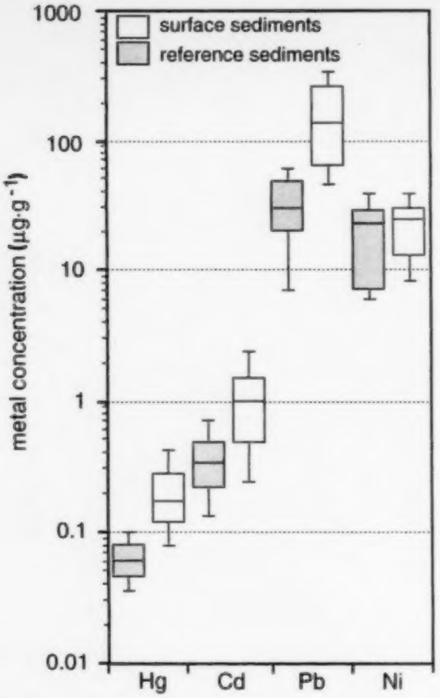


FIG. 2. Box-and-whisker plot of heavy metal concentrations in surface and reference sediments, showing medians, upper and lower quartiles, and the 10th and 90th percentiles (surface sediments: 0–1 cm depth; reference sediments: 20–30 cm depth; $n = 85$).

the regression line, we could estimate values for the censored data using their normal scores. This method, rather than using simple substituting methods, reduces estimation errors substantially for summary statistics.

Because of the many intercorrelations in our data set, the structure of the path models and candidates for predictor variables could not be selected solely on the basis of their correlations with lake sediment concentrations. Such intercorrelations may either mask the effects of important causal factors or be the source of spurious correlations. Our strategy for the model building was therefore to use a priori knowledge to establish the basic structure of the path models. We then inspected the solutions, and if necessary, we reestimated the models by excluding or including other scientifically plausible variables.

In a path model, the effects that a predictor variable has on a criterion variable are expressed by its path coefficients. They do not express the rate of change in terms of its original measurement units, but in standard deviations. They are therefore useful as a relative measure for comparing the magnitude of the effects that different predictor variables have on a criterion variable. The effect of a predictor variable may be both direct and indirect. The indirect effect along a path equals the product of the path coefficients. The total effect is the sum of direct and indirect effects. For an introduction to path analysis, see Sokal and Rohlf (1981) or Kingsolver and Schemske (1991) and references therein. The results from path analyses are customarily presented in path diagrams, where the effects of predictor variables are depicted by single-headed arrows pointing to the criterion variables. Unanalyzed correlations are usually depicted by double-heated arrows. However, to avoid visual cluttering, we have omitted such double-headed arrows in our path diagrams.

In a simple path model with unidirectional effects (recursive model) and directly observed variables, the path coefficients are the standardized regression coefficients from the structural equations representing the model. Traditionally, path analysis for such a model is done by a series of standardized multiple regressions. An alternative approach is to consider the model as a system of equations and estimate all the path coefficients simultaneously. This is done by causal modelling programs, such as LISREL (Jöreskog and Sörbom 1989), using a maximum likelihood method to calculate the model parameters from the correlation or covariance matrix. The program estimates several diagnostic measures of fit that can be used to interpret how well a model fits the data. Because of these advantages, we used LISREL for our path analyses. However, path analyses are subject to many of the same assumptions as other general linear models. We therefore also made standard regression analyses of the structural equations representing the path models, inspecting the residuals to ensure normality, linearity, and homeoscedatisity, and studying leverage plots to avoid influence from outliers. For the exploratory data analyses and the regressions, we used the statistical program JMP (SAS Institute Inc. 1991).

## Model Formulation

From the references given in the introduction, we knew we could expect Hg, Pb, and Cd to be associated with humic matter in the lake sediments. We therefore treated the dose variables and $OC_s$ as a high-priority block of variables in our causal modelling. pH and lake depth may also be of vital importance. The mobility of Cd and Ni increases during acidification (Bergkvist et al. 1989), and they are released from their binding sites on organic and inorganic matter. A weak opposite effect of pH has been reported for Hg whereas Pb seems to be more unaffected (Schindler et al. 1980). Lake depth may influence sediment concentrations due to sedimentation processes such as size-selective sorting and redistribution of particles. We assume the depth effect to be greatest for elements associated with fine organic particles.

The areas of the catchment and lake surface, or the ratio between them, are also variables of interest, as they will weight the contributions of direct deposition to the catchment area and lake surface. These variables, however, together with lake depth form a highly intercorrelated group. Uncritically used, they will introduce undesirable multicolliniarity or even singularity in the models. A logarithmic plot of lake surface area versus catchment area revealed that the ratio between the two diminished with increasing catchment area. By excluding lake area from the variable set, we removed the problems of singularity and multicolliniarity, but this leads to some ambiguity in the interpretation the effects of lake depth and catchment area.

Further, we expected that lake depth and pH, together with TOC, could be used to explain some of the variation in $OC_s$. The correlation matrix also indicated that lakes with large catchment areas ($\log A_c$) had low $OC_s$ values ($r = -0.55$). We included this variable in the analysis, as we believe there are important causal mechanisms behind this correlation.

Based on these rationales, we formulated the basic structure of our models: the dose variables (moss concentrations and

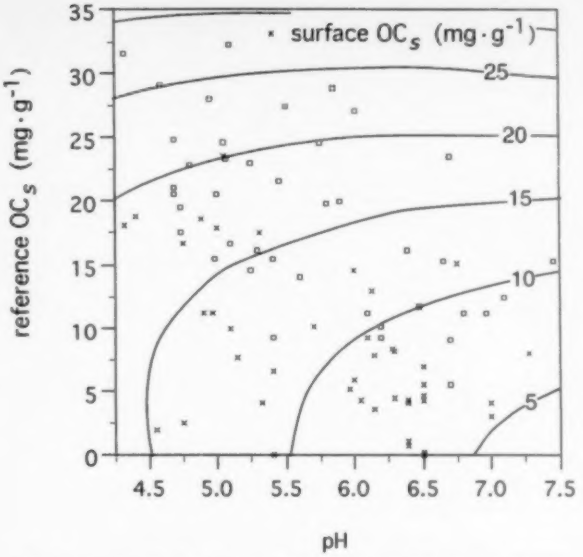


FIG. 3. Contour plot of organic carbon in surface sediments (surface OC$_s$) in a quadratic response model. Independent variables are organic carbon in reference sediments (reference OC$_s$) and pH in lake water. The crosses indicate lakes where OC$_s$ have increased towards the surface, and the squares indicate lakes where OC$_s$ have decreased. $R^2 = 0.66$, df = 5/79, $F = 30.28$, $p < 0.0001$.

overbank sediment concentrations), together with lake depth and pH (except for Pb), should be candidates for predictor variables with direct effects on the lake sediment concentrations. OC$_s$ also should be a variable with direct effects, but we allowed it to be affected by other variables in the model (TOC, pH, lake depth, and catchment area). After the first model runs, we inspected the solutions. If necessary, we reestimated the models, leaving out insignificant effects or including other plausible predictor variables. The correlation matrix for the data set given in Table 2 will enable others to analyze our data.

## Results

### Surface Sediments versus Reference Sediments

In general, the concentrations of Hg, Pb, and Cd were substantially higher in surface sediments than in reference sediments: the interquartile ranges showed no overlap, and the median values were elevated by a factor of 3.0, 4.6, and 2.9, respectively (Fig. 2). The situation was different for Ni. We could only find a small increase in the median Ni concentration (a factor of 1.1), and the mean difference between surface and reference values was at the borderline of significance ($\Delta Ni_s = 2.3$, SE = ± 1.3 mg·g$^{-1}$, $t = 1.88$, df = 84, $p = 0.06$).

The strong increase in the concentrations of Hg, Pb, and Cd was not associated with a general increase in the content of organic matter in sediments (OC$_s$), as we could only observe a small, insignificant difference between reference and surface sediments for OC$_s$ (mean $\Delta OC_s = 0.09 \pm 5.59$ mg·g$^{-1}$). However, a closer inspection of the data revealed a distinct pattern: OC$_s$ in the surface sediments had increased considerably in acid lakes with low OC$_s$ values in reference sediments, but decreased in more neutral or acidic lakes with high reference OC$_s$. This first category of lakes consists of mainly acidified nonhumic oligotrophic lakes whereas the others have a more dystrophic character. In a contour plot the lakes with an increase in OC$_s$ were located below a diagonal line running from lakes with low pH and high reference OC$_s$ to lakes with high pH and low reference OC$_s$ (Fig. 3). A multiple regression analysis showed pH together with reference-OC$_s$ to explain 65% of the variation in OC$_s$ (Table 3). The pattern of $\Delta OC_s$ seemed not to be influenced by Fe concentrations in sediments. We found no general increase in the Fe concentrations in surface sediments compared with reference sediments (mean $\Delta Fe_s = 4.2 \pm 57.7$ mg·g$^{-1}$). There was no significant correlation between $\Delta Fe_s$ and $\Delta OC_s$ ($r = -0.12$), and Fe$_s$ therefore could not be used as an explanatory variable in the regression model.

TABLE 3. Multiple regression of OC$_s$ in surface sediments on organic carbon in reference sediments (reference OC$_s$) and pH ($n = 85$, $R^2 = 0.65$, $F_{2,82} = 75.40$, $p < 0.0001$).

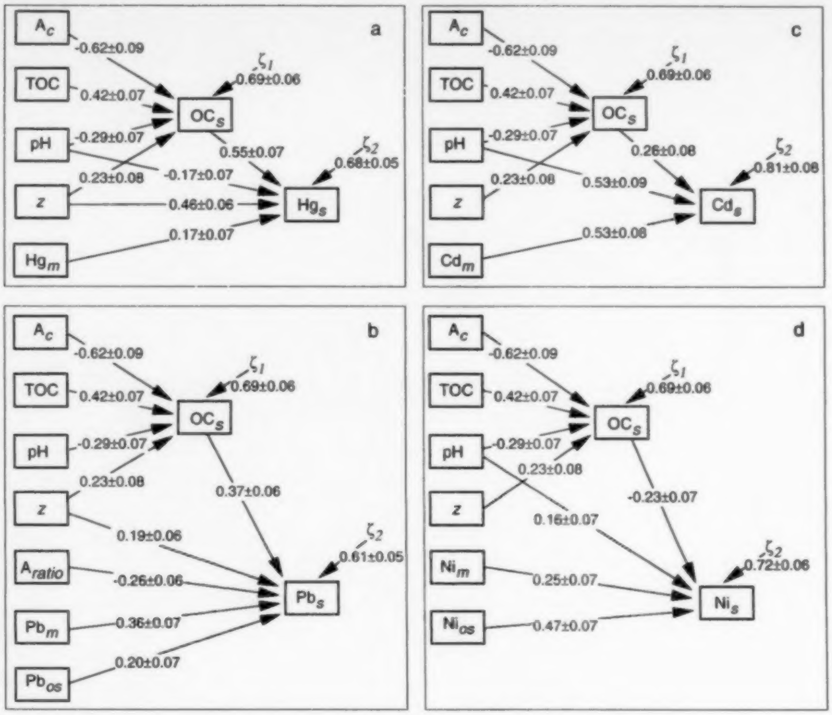
| Term | Coefficient | SE | $t$ | $p$ |
|---|---|---|---|---|
| Intercept | 21.17 | 4.32 | 4.90 | <0.0001 |
| pH | −2.50 | 0.66 | −3.76 | 0.0003 |
| Reference OC$_s$ | 0.50 | 0.06 | 8.31 | <0.0001 |

### Hg Model

The basic model for Hg was a model with direct effects of the atmospheric deposition (i.e., moss concentration: log Hg$_m$), OC$_s$, pH, and lake depth (log $z$) and indirect effects of log TOC, pH, lake depth, and catchment area (log $A_c$) through OC$_s$. This is a recursive model with two endogenous variables, four exogenous variables, and two random error variables ($\zeta_n$) (Fig. 4a). The error variables are composite variables, including all residual causes associated with the endogenous variables.

The model run confirmed our hypotheses. The parameter estimates (path coefficients) had the expected signs and the standard errors were of acceptable size, although the standard errors of the direct effect of pH on log Hg$_s$ indicated borderline significance. The total coefficient of determination was good ($R^2 = 0.69$). A small chi-square test statistic ($\chi^2 = 0.40$, df = 3, $p = 0.94$) indicated a model that adequately describes the data. However, modification indices suggested that the solution would be slightly improved by allowing a minor effect of Fe in sediments (log Fe$_s$) on log Hg$_s$. We are aware that there may be causal mechanisms behind this effect, but we did not include this variable in our model, as its interpretation may be dubious. We will discuss this later.

Overall, the effect of the Hg deposition rate (log Hg$_m$) was small compared with the effect of other variables. There were no indirect effects of log Hg$_m$; its total effect was therefore identical to its path coefficient (Fig. 4a). Thus, a change of 1 standard deviation unit in log Hg$_m$ results in a change of 0.17 standard deviation unit in log Hg$_s$. The variable with the strongest direct effect on log Hg$_s$ was OC$_s$, with a path coefficient of 0.55. However, much of the effect of OC$_s$ is due to the indirect effects of four other variables (TOC, lake depth, pH, and $A_c$). Lake depth (log $z$) was therefore the exogenous variable with the greatest total effect.


a
A_c
TOC
pH
z
Hg_m
OC_s
Hg_s
ζ1
ζ2
-0.62±0.09
0.42±0.07
-0.29±0.07
0.23±0.08
0.69±0.06
0.55±0.07
-0.17±0.07
0.68±0.05
0.46±0.06
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c
A_c
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pH
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Cd_m
OC_s
Cd_s
ζ1
ζ2
-0.62±0.09
0.42±0.07
-0.29±0.07
0.23±0.08
0.69±0.06
0.26±0.08
0.81±0.08
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b
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OC_s
Pb_s
ζ1
ζ2
-0.62±0.09
0.42±0.07
-0.29±0.07
0.23±0.08
0.69±0.06
0.37±0.06
0.19±0.06
0.61±0.05
-0.26±0.06
0.36±0.07
0.20±0.07
d
A_c
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pH
z
Ni_m
Ni_os
OC_s
Ni_s
ζ1
ζ2
-0.62±0.09
0.42±0.07
-0.29±0.07
0.23±0.08
0.69±0.06
-0.23±0.07
0.16±0.07
0.72±0.06
0.25±0.07
0.47±0.07

FIG. 4. Path diagrams of the sediment models. Path coefficients with standard errors are printed on the arrows. See Table 1 for explanations of abbreviations.

It had a direct effect of 0.46 and an indirect effect of 0.13, resulting in a total effect of 0.59 ± 0.09. The total effect of the remaining exogenous variables decreased in the following order: log $A_c$ (−0.34 ± 0.07); pH (−0.33 ± 0.08); log TOC (0.23 ± 0.05).

Results for multiple regressions of the structural equations are given in Table 4, as the unstandardized regression coefficients are useful for prediction purposes. The determination coefficients ($R^2$) showed that the model explained 52% of the variation in $OC_s$ ($F = 34.77$, df = 4/127, $p < 0.0001$) and 54% of the variation in log $Hg_s$ ($F = 37.05$, df = 4/127; $p < 0.0001$). The parameter estimates for the structural equation describing $OC_s$ are identical for all the following models. In Fig. 5a, we show the estimated $Hg_s$ values at different Hg deposition rates and pH values.

## Pb Model

Our basic Pb model was identical to the Hg model, with the exception of no direct effect from pH. The first model run confirmed our hypotheses, yet modification indices strongly suggested that the sediment concentration (log $Pb_s$) was also influenced by the catchment area ratio (log $A_{ratio}$). A reestimation of the model confirmed this; an increase in this ratio was associated with a decrease in log $Pb_s$. However, modification indices now indicated that a small improvement could be achieved by allowing for an effect of Fe in sediments (log $Fe_s$). As in the case for Hg, we did not want to incorporate this variable in our model.

The model (Fig. 4b) was generally well fitted ($\chi^2 = 2.57$, df = 6, $p = 0.86$) with a high total coefficient of determination ($R^2 = 0.76$). The parameter estimates had expected signs and acceptable standard errors. In general, Pb behaved in a very similar way as Hg. The main differences were the weaker association with $OC_s$ (path coefficient: 0.37) and the negative effect of the catchment area ratio (log $A_{ratio}$). The moss concentration (log $Pb_m$) was the most influential exogenous variable (path coefficient: 0.36), being almost twice as large as the geochemical conditioned input (log $Pb_{obs}$) (0.20). The total effects of log $z$, log $A_l$, and log $A_{ratio}$ were of approximately same absolute magnitude (0.28 ± 0.07, −0.23 ± 0.05, and −0.26 ± 0.06, respectively) whereas those of log TOC and pH were somewhat lower (0.16 ± 0.04 and −0.11 ± 0.03). The regression analysis (Table 4) explained 62% of the variation in log $Pb_s$ ($F = 41.53$, df = 5/126, $p < 0.0001$). In Fig. 5b, we show estimated $Pb_s$ values at different Pb deposition rates and pH values.

## Cd Model

The basic model of Cd was equivalent to that of Hg. We allowed for direct effects of the deposition rate (i.e., moss concentration: log $Cd_m$), $OC_s$, log $z$, and pH on sediment concentration (log $Cd_s$). The only difference was that we expected pH to positively affect log $Cd_s$, as Cd belongs to a group of metals mobilized by acidification. The first model run showed all effects except that of log $z$ to be significant. We then reestimated the model without direct effects of $z$ (Fig. 4c). This model was well fitted ($\chi^2 = 5.19$, df = 4, $p = 0.27$), with a high total coefficient of determination ($R^2 = 0.68$). All parameter estimates had expected signs and acceptable standard errors. Inspections of modification indices

TABLE 4. Multiple regression analyses of the structural equations representing the path models ($n = 132$).

| Dependent variable | Term | Coefficient | SE | $t$ | $p$ |
|---|---|---|---|---|---|
| $OC_s$ | Intercept | 29.816 | 4.246 | 7.02 | <0.001 |
| | log TOC | 7.030 | 1.154 | 6.09 | <0.001 |
| | pH | −2.806 | 0.679 | −4.13 | <0.001 |
| | log $z$ | 4.495 | 1.681 | 2.67 | 0.008 |
| | log $A_c$ | −4.736 | 0.687 | −6.89 | <0.001 |
| log $Hg_s$ | Intercept | 0.874 | 0.444 | 1.97 | 0.051 |
| | log $Hg_m$ | 0.495 | 0.192 | 2.56 | 0.011 |
| | $OC_s$ | 0.019 | 0.003 | 7.74 | <0.001 |
| | pH | −0.059 | 0.024 | −2.46 | 0.015 |
| | log $z$ | 0.318 | 0.044 | 7.27 | <0.001 |
| log $Pb_s$ | Intercept | 0.974 | 0.142 | 6.87 | <0.001 |
| | log $Pb_m$ | 0.460 | 0.090 | 5.11 | <0.001 |
| | log $Pb_{os}$ | 0.232 | 0.084 | 2.77 | 0.006 |
| | $OC_s$ | 0.016 | 0.003 | 5.95 | <0.001 |
| | log $_z$ | 0.164 | 0.049 | 3.32 | 0.001 |
| | log $A_{ratio}$ | −0.175 | 0.038 | 4.61 | <0.001 |
| log $Cd_s$ | Intercept | −1.682 | 0.569 | −2.96 | 0.004 |
| | log $Cd_m$ | 1.065 | 0.160 | 6.09 | <0.001 |
| | $OC_s$ | 0.015 | 0.005 | 3.20 | 0.002 |
| | pH | 0.297 | 0.049 | 6.85 | <0.001 |
| log $Ni_s$ | Intercept | 0.289 | 0.228 | 1.27 | 0.207 |
| | log $Ni_m$ | 0.372 | 0.095 | 3.92 | <0.001 |
| | log $Ni_{os}$ | 0.557 | 0.083 | 6.72 | <0.001 |
| | $OC_s$ | −0.010 | 0.003 | −3.11 | 0.002 |
| | pH | 0.072 | 0.034 | 2.13 | 0.035 |

indicated that the model would not be substantially improved by allowing for other effects. Cd in sediments (log $Cd_s$) seemed to be less influenced by $OC_s$ than Hg and Pb (path coefficient: 0.23). The most influential variable was log $Cd_m$, with a path coefficient of 0.53. pH also had a strong direct positive effect on log $Cd_s$ (0.53), but this was somewhat counteracted by its negative effect through $OC_s$, resulting in a total effect of 0.46 ± 0.08. The total effect of the remaining exogenous variables decreased in the following order: log $A_c$ (−0.16 ± 0.06); log TOC (0.11 ± 0.04); log $z$ (0.06 ± 0.03). The regression analysis (Table 4) showed the predictor variables to explain 34% of the variation in log $Cd_s$ ($F = 21.49$, df = 3/128, $p < 0.0001$). In Fig. 5c, we show estimated $Cd_s$ values at different Cd deposition rates and pH values.

### Ni Model

As we expected Ni to behave in a similar manner as Cd, except for a diluting effect of $OC_s$, our basic model was equivalent to what we arrived at for $Cd_s$. The path analysis confirmed this. All parameter estimates were significant and had expected signs (Fig. 4d). Although the total coefficient of determination was good ($R^2 = 0.71$), the $\chi^2$ statistic was high ($\chi^2 = 11.94$, df = 5, $p = 0.04$), indicating a bad fit. This was probably due to a correlation between the residuals of log $Ni_m$ and $OC_s$, as condition indices suggested log $Ni_m$ to have a negative effect on $OC_s$. This is unlikely, and we believe this to be due to a spurious correlation. We therefore regard the model to adequately describe the most important causal relationships.

The path coefficient of overbank sediments (log $Ni_{os}$) was almost twice as large as that of moss concentration (log $Ni_m$) (0.47 versus 0.25). These were the two most influential variables. The effect of pH on log $Ni_s$ was much weaker than for log $Cd_s$. The direct effect of pH was 0.16 whereas the indirect effect through $OC_s$ was 0.07, giving a total effect of 0.23 ± 0.07. The positive indirect contribution of pH was a result of the association of Ni with inorganic sediments; the path coefficient of $OC_s$ was therefore negative (−0.23). The total effect of the remaining exogenous variables decreased in the following order: log $A_c$ (0.14 ± 0.05); log TOC (−0.10 ± 0.04); log $z$ (−0.05 ± 0.02). The regression analysis (Table 4) shows the predictor variables to explain 50% of the variation in log $Ni_s$ ($F = 31.26$, df = 4/127, $p < 0.0001$). In Fig. 5d, we show estimated $Ni_s$-values at different Ni deposition rates and pH values.

## Discussion

In this study, we show that a pronounced increase has occurred in the concentrations of Hg, Pb, and Cd in surface sediments of lakes in southern Norway, as compared with sediments deposited in preindustrial times. Typically, the concentrations have been raised by a factor of 3–5. In the case of Ni, such an increase was found only in lakes in the vicinity of smelters, and in general we registered only an insignificant elevation of the Ni concentrations. Our results from the path models indicate that elevated concentrations of these heavy metals are mainly a result of increased atmospheric deposition modified by acidification-related processes.

The long-term deposition of acidifying $SO_x$ and $NO_x$ has lead to severe disturbances of the aquatic environment in the acidification-sensitive areas of southern Norway (Henriksen et al. 1988, 1989). Apart from altering the mobility of several

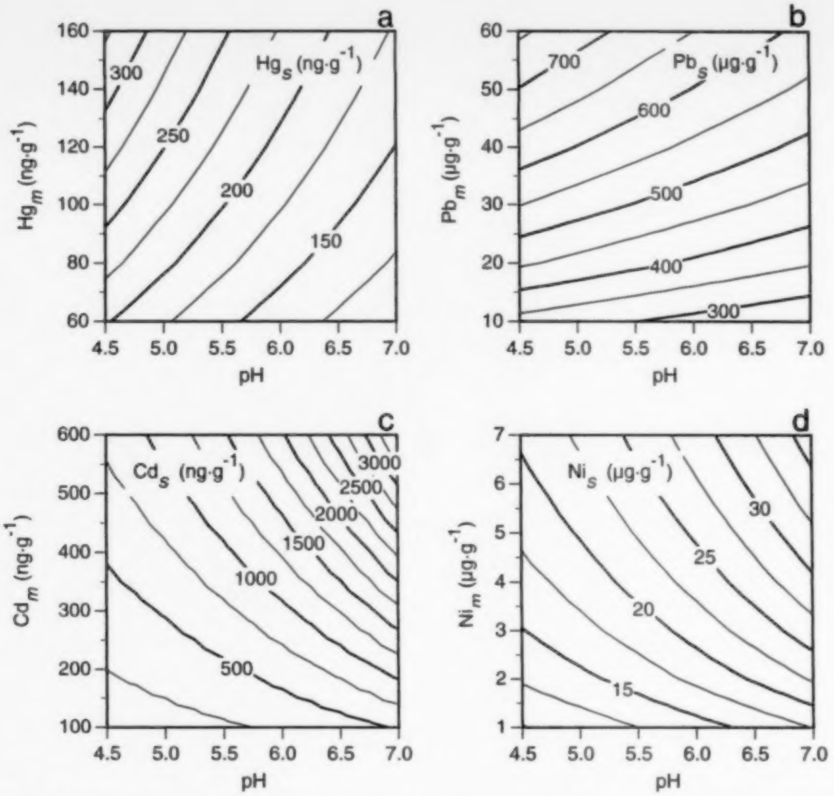

FIG. 5. Contour plot of estimated surface sediment concentrations of Hg, Pb, Cd, and Ni at different moss concentrations and pH. The moss concentrations are used as a relative measure of the atmospheric deposition rates. The estimates are based on the equations in Table 4. All other predictor variables are kept constant, equal to sample medians.

metals in soils and aquatic sediments, with the potential damage this may have to biota, some of the most pronounced effects of acidification are an increased sedimentation rate and reduced decomposition of organic matter (Traaen 1980; Schindler et al. 1992). This may lead to a reduction in dissolved organic matter (DOC) in lake water and an increase in the organic component of surface sediments. A major fraction of DOC in freshwater is composed of refractory allochthonous humic substances. These are effective metal-complexing agents, and they are believed to play a fundamental role in the transport of certain metals to lake water and sediments. Thus, $OC_s$ is an important variable in our path models, and therefore, we will first discuss the factors affecting $OC_s$ before turning to the individual metal models.

### Factors Affecting $OC_s$

The distinct pattern of $OC_s$ in Fig. 3 may indicate that the organic fraction in sediments had been altered in two ways. First, there seemed to be an acidification-related increase in surface $OC_s$ as compared with reference sediments. Second, we found that this difference ($\Delta OC_s$) had a tendency to be negative in lakes with high values of $OC_s$ in reference sediments (lakes with a more dystrophic character). We find such a decrease somewhat surprising, as we expected in general to find slightly elevated $OC_s$ values in surface sediments because of the effects of microbial degradation of subsurface sediments and an acidification-related increase in surface $OC_s$. Evidently this decrease could not be due to a simple displacement of organic matter by ion hydroxides migrating upwards along a redox gradient in the sediments, as there was no correlation between $\Delta OC_s$ and $\Delta Fe_s$. Other diagenetic or deposition processes must therefore have caused this reduction. Although numerous factors may be involved, and our study has not been designed to specifically address these questions, we would like to point out some factors recently discussed. Acidification of soils may cause a reduction in the water solubility of humic matter (Hay et al. 1985), resulting in a stronger retention of humic matter in acidified catchments and a decline in lake DOC (De Haan 1992; Schindler et al. 1992). Also, climatic changes may produce changes in the runoff of DOC from the catchments (Forsberg 1992; Schindler et al. 1992). If such a decline in allochthonous DOC has occurred, it may result in a lower $OC_s$ in dystrophic lakes whereas acidified clear-water lakes dominated by autochthonous DOC may have increased in $OC_s$ due to reduced microbial degradation and increased sedimentation. Whether these factors have been involved in the production of the observed $\Delta OC_s$ pattern remains unclear, but we would like to see this phenomenon studied further, as it may have significant implications for the biogeochemistry of several elements.

By allowing a negative effect of pH on $OC_s$ in our models, we have incorporated an acidification-related increase in the organic fraction in sediments. Additionally, we have let TOC, lake depth, and catchment area influence $OC_s$. Whereas TOC may be looked upon as the source variable for $OC_s$, lake depth may be interpreted as a variable involved in sedimentation and sorting of organic matter. The retention of fine organic particles and humic aggregates will be greater in deep lakes than in more shallow ones with short water residence times. Moreover, resuspension and resedimentation of sediment will cause a continual sorting and transportation of fine-graded particles from the shallow areas to the deeper parts of the lake (the funneling effect: Ohle 1962; Håkanson and Jansson 1983).

The interpretaton of the strong negative effect of catchment area on $OC_s$ is more subtle, as there are obviously factors associated with it, and not the catchment area per se, that influence $OC_s$. Evidently, this effect is not due to other area-correlated characteristics such as lake area or the ratio of catchment area to lake area, as these variables could not significantly enter the models. However, without discussing this effect in depth, we suggest that it may partly be explained by increased input of inorganic sediments from rivers draining large catchment areas, as the greater stream velocity in these rivers increases the fluvial erosion and keeps larger minerogenic particles in suspension. For a discussion of the humic content in lake water and its relationship to watershed and lake morphometry, see Rasmussen et al. (1989).

Kerndorff and Schnitzer (1980) studied the sorption of various metals on humic acids, and for the metals treated here, they found the following order of complex stability: $Hg \geq Pb > Cd > Ni$. This is the same order that we find when we compare the effects of $OC_s$ on sediment metal concentrations in our models, and we therefore believe that $OC_s$ satisfactorily reflects the association of metals with humic matter in sediments. Kerndorff and Schnitzer (1980) also found that the sorption of Hg to humic acids is insensitive to changes in pH (almost 100% throughout the studied pH range of 2.8–5.8). This was also the case for Pb, except for a somewhat weaker sorption efficiency at lower pH values ($\leq 3.7$). Cd and Ni were weakly sorbed and were sensitive to changes in pH throughout the studied range. In our models, we found a similar pattern; Cd and Ni were mobilized by acidification, while Hg and Pb were rather insensitive to acidification.

### Hg and Pb Models

For metals with strong affinity to humic matter, but not easily mobilized by acidification, acidification may reinforce the effects of increased atmospheric depositions on sediment concentrations. This is well demonstrated in our Hg and Pb models. Both metals complex readily with organic ligands and are therefore closely connected to the carbon cycle of lakes (Santschi 1988). Hg and Pb enter lakes through direct deposition and in runoff from catchment areas. Budget studies of forest ecosystems in Sweden and Germany, summarized by Bergkvist et al. (1989) and Johansson et al. (1991), show a major part (70–95%) of atmospherically deposited Hg and Pb to be accumulated in soils whereas the release to the aquatic environment is largely controlled by the amount of dissolved humic matter. Factors affecting input of humic matter and the carbon cycle within lakes will therefore inevitably influence sediment concentrations of Hg and Pb. We therefore suggest that the strong effect of $OC_s$ on the sediment metal concentrations in our models reflects the role of humic matter in the transport of Hg and Pb from the catchments, and probably also the scavenging of dissolved ions by settling humic aggregates (Santschi 1988).

We found negative correlations between pH of lake water and the dose variables log $Hg_m$ and log $Pb_m$ ($r = -0.35$ and $-0.44$, respectively). These match well with the reported correlations between $SO_x$, Hg, and Pb concentrations for the southern stations in the Nordic precipitation network (Iverfeldt 1991; SFT 1991). These correlations are assumed to reflect both the concomitant emissions of $SO_x$ and heavy metals in the source areas on the European continent and the positive net effect of acidifying pollutants on the transformation of gaseous $Hg^0$ to the oxidized $Hg^{2+}$ found in cloud droplets and rain (Brosset and Lord 1991). In our Hg model, we have allowed pH to influence $Hg_s$ in two ways: one direct effect and one indirect through $OC_s$. We believe that the indirect effect of pH is caused by increased sedimentation and decreased mineralization of DOC during acidification, leading to an increase of Hg-enriched humic matter in surface sediments. The weak direct effect reflects the fact that Hg in situ, in contrast with most other metals, is more strongly adsorbed to humic matter during acidification (Schindler et al. 1980; Lodenius et al. 1983). Thus, the coincident deposition of Hg and acidifying compounds makes lake sediments very liable to Hg enrichment during acidification. We could not detect any direct effects of pH on $Pb_s$, which is in accordance with field studies concluding that acidification has no significant effect on the adsorption of Pb to humic matter in forest soils (Bergkvist et al. 1989) or in lake sediments (Schindler et al. 1980; Norton 1986). This, and the weaker association of Pb to humic matter, makes $Pb_s$ somewhat less sensitive than $Hg_s$ to the effects of acidification. One may see this in Fig. 5, following the trajectory of $Hg_s$ and $Pb_s$ in a hypothetical lake as it moves from an unpolluted towards a more polluted and acidified state.

Also, we believe that the negative effect of the ratio of catchment area to lake area in our Pb model is due to the adsorption of Pb to humic substances in soils. Direct deposition on the surface is therefore an important pathway for Pb entering lakes. This is also true for Hg (Johansson et al. 1991), but we suggest that reemissions of gaseous $Hg^0$ from soils and lake surfaces (Vandal et al. 1991; Rada et al. 1993) together with a pH-mediated alteration of its mobility may conceal an effect of area ratios.

The direct effect of lake depth on both $Hg_s$ and $Pb_s$ indicates that the quality of organic material in sediments changes with depth and becomes more enriched with these metals. Such an effect may be caused by a sorting of organic matter, where deeper lakes have a more fine-graded organic material enriched with Hg and Pb. Sediments in shallow lakes are probably more influenced by vegetation originating from the littoral zone and the sediment surface. This organic material probably contains less Pb and Hg than allochthonous humic matter.

Modification indices in our models suggest that Fe in sediment could have a small influence on $Hg_s$ and $Pb_s$. However, we did not include this effect in our models because we believe that it may be of a spurious rather than causal nature. An enrichment of Fe is commonly found in surface waters with high DOC, probably due to complexion to humic matter (Stumm and Morgan 1981; Urban et al. 1990; Morel and

Hering 1993). Thus, in sediments dominated by allochthonous humic matter, there may be a spurious correlation between Fe and other heavy metals with a strong affinity to organic ligands. Besides, adsorption of newly formed ferric hydroxides to the surface of humic colloids results in their decharging and flocculation (Wetzel 1975; Urban et al. 1990). We suggest that this process could also contribute to a simultaneous enrichment of Fe and heavy metals in sediments.

The path coefficients of the dose variables $Hg_m$ and $Pb_m$ suggest that $Pb_s$ is more influenced by atmospheric deposition than $Hg_s$. This seems reasonable considering the transformation of deposited $Hg^{2+}$ to gaseous $Hg^0$. The lack of an estimate of the geochemical contribution of Hg is probably of less importance for the adequacy of our model, as atmospheric deposition is considered to be the dominating source for Hg entering freshwater ecosystems in this region (Johansson et al. 1991). Pb is a far more abundant metal in the earth's crust; thus, the geochemical contribution of this element was significant, although considerably smaller than the atmospheric contribution.

### Cd and Ni Models

The Cd and Ni models showed these metals to behave quite differently from Hg and Pb. Cd seemed to be associated with the organic fraction in sediments, but to a lesser extent than Hg and Pb. The most important difference, however, was the strong direct positive effect of pH on $Cd_s$, indicating that particle-bound Cd is mobilized to the water phase by acidification. These results are in concordance with those of Tessier et al. (1993) studying sediments from lakes in Quebec and Ontario. Their results indicated that Cd was sorbed mainly to sedimentary organic matter and that the binding intensity (equilibrium constant) was very sensitive to pH variations. Also, Borg and Andersson (1984) demonstrated by in situ dialysis of acidified lake water that the truly dissolved fraction (mainly free ionic species and inorganic complexes) was about 60–100% at pH 4.5–6.0 whereas this fraction decreased to 10–60% at pH 6.0–7.0. This pH-dependent sorption chemistry will also have serious consequences for the scavenging effect of organic particles settling through a lake and for the release of Cd from the catchment. Studies from Sweden (Bergkvist 1987) and Germany, summarized by Bergkvist et al. (1989), show that acidified forest catchments may have a net loss of Cd. Cd accumulated in organic soils seems to be desorbed from humic matter when the acid neutralizing capacity (ANC) of the soil is depleted, with the result that the input to lakes may be greater than the present deposition rates alone should indicate.

We found atmospheric deposition of Cd (i.e., moss concentrations: log $Cd_m$) to be the variable with the strongest effect on sediment concentrations, closely followed by pH. Atmospheric deposition had comparable effects in no other models. The deposition was negatively correlated with pH of lake water ($r = -0.41$) and positively correlated with $Hg_m$ and $Pb_m$ ($r = 0.51$ and 0.78, respectively). This indicates that the Cd, Hg, and Pb deposited in southern Norway originate mainly from the same European source areas as do anthropogenic $SO_x$ and $NO_x$. This is in accordance with results from air concentration studies (Amundsen et al. 1992). Again, a geochemical contribution is of minor importance for our model. Cd is a rare metal in crustal rocks, and weathering of indigenous rocks is likely to be of little significance for the Cd levels in Scandinavian surface waters (Steinnes 1990).

The co-deposition of Cd and acidifying compounds implies that increased deposition is associated with a shift in the speciation towards truly dissolved Cd species. This may have an adverse effect on aquatic biota, as it leads to an increased flux of Cd from the catchments to the lake ecosystems and favours formation of the most toxic and bioavailable Cd species (free aquo ions). Also, if we accept that there is a statistical association between the deposition rates and lake acidification, then increased deposition is not followed by an increase in the sediment concentrations equivalent to that indicated by the path coefficient between $Cd_m$ and $Cd_s$, as this effect will be somewhat counteracted by a pH-mediated shift in speciation. One may see this in Fig. 5, following the trajectory of $Cd_s$ in a hypothetical lake as it moves from an unpolluted towards a more polluted and acidified state.

In our Ni model the path coefficient between $OC_s$ and log $Ni_s$ indicated that this element was mainly associated with the inorganic sediment fraction. Thus, the organic component of sediments may be regarded as a bulk material diluting the concentrations of Ni. However, the strength of this effect was low (path coefficient of $OC_s$: $-0.18$), which could mean that significant amounts of Ni were also present in the organic fraction. Such a partitioning has been reported by Brezonik et al. (1990) who found Ni to be significantly present in both the organic and inorganic (metal hydroxide) sediment fractions in an experimentally acidified seepage lake.

We also found Ni to be mobilized by acidification, but the effect of pH was considerably weaker than in the case of Cd. The weak effect of pH may at first sight seem incomprehensible, as the solubility of Ni in soils is regarded to be very sensitive to pH (Bergkvist et al. 1989 and references therein). However, the strong effect of geochemically conditioned Ni indicates that significant amounts of minerogenic Ni occur in the inorganic particle fraction, probably in the form of minerals not easily mobilized by lake acidification.

Generally, the atmospheric deposition of Ni seemed to be of secondary importance as compared with the geochemically conditioned input (the path coefficient of log $Ni_m$ was approximately 50% smaller than that of log $Ni_{os}$), and accordingly, Ni was the element showing the lowest contamination factor. One of the main sources of atmospheric Ni in the Nordic countries are emissions from copper–nickel smelters (Rühling et al. 1987). The Ni emissions from this industry are probably mainly associated with larger particles deposited near the smelters, and we have previously shown the pattern of $Ni_s$ in Norway to be greatly influenced by such local sources (Rognerud and Fjeld 1993; Rognerud et al. 1993). The sediment concentrations of Ni are therefore poorly correlated with the concentrations of the other metals studied and to lake pH.

In summary, our study shows that the joint effects of man-made deposition of heavy metals and acidification may critically influence the metal accumulation in lake sediments. Also, it emphasizes the important role of dissolved humic matter in the transport of certain metals from the catchments to lake sediments. For a better understanding of the cycling of these metals, there is a need of more knowledge on how acidification of soil and lake water affects the accumulation of organic carbon in sediments.

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# Determining Fish Age from Temporal Signatures in Growth Increments[1]

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The temporal signature technique can be used to assign age to fish that show an incomplete or indistinct growth history at the margins of their scales. The temporal signature technique matches part of an individual's "environmental" growth history to characteristic patterns found in a master chronology that was developed from reliably aged specimens of a species in a particular environment. An error sum of squares measures the concordance between an individual's environmental growth history and the master chronology. Ages assigned to walleye (*Stizostedion vitreum*) by the temporal signature technique and by examination of scales were compared to assess the performance of the new technique. Scale-age agreed with one of the three most likely signature-ages in 67–77% of the comparisons using all observed increments. These results are purposely conservative because of the methods employed and the nature of the example. All observed growth increments should be used in applying the temporal signature technique, but age may still be accurately assigned if as few as three increments are available. The temporal signature technique will perform best for species that exhibit high interannual variation in growth.

On peut recourir à la technique de la signature temporelle pour déterminer l'âge de poissons qui présentent un registre de croissance incomplet ou indistinct aux bordures des écailles. Cette technique consiste à faire correspondre une partie du registre de croissance « environnemental » de l'individu à des configurations caractéristiques retrouvées dans une chronologie maîtresse établie à partir de spécimens d'une espèce dont les âges ont été déterminés avec certitude pour un milieu donné. Une somme des carrés des résidus mesure la concordance entre le registre de croissance environnemental de l'individu et cette chronologie maîtresse. On a comparé les âges attribués au doré (*Stizostedion vitreum*) au moyen, d'une part, de la technique de la signature temporelle et, d'autre part, de l'examen des écailles afin d'évaluer la performance de la nouvelle technique. L'âge déduit des écailles concorde avec un des trois plus probables âges déduits de la signature temporelle dans 67 à 77 % des comparaisons utilisant tous les incréments observés. On fait preuve d'une prudence volontaire vis-à-vis de ces résultats en raison des méthodes employées et de la nature de l'exemple. Tous les incréments de croissance observés devraient être utilisés lorsqu'on applique la technique de la signature temporelle, mais l'âge peut encore être déterminé avec précision à partir de seulement trois. La technique de la signature temporelle donnera ses meilleurs résultats pour les espèces dont la croissance montre de fortes variations interannuelles.

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We propose a method to correctly assign age to fish from a partial growth history on scales or other calcified structures. Slow growth of older fish or resorption of previously deposited scale tissue frequently leads to underestimation of age from scales (Simkiss 1974; Beamish and McFarlane 1987; Casselman 1987). Nevertheless, the innermost regions of the scale (i.e., proximal to the focus) contain an indelible and distinguishable record of at least the first few years of an individual fish's growth. For example, a 20-yr-old fish showing eight distinct annuli before cessation of incremental scale growth contains a valid growth history of the first 8 yr of its life. In principle, this is the same record that an 8-yr-old would show had it been taken 12 yr before the 20-yr-old was taken. This paper describes a new technique, the temporal signature, that can be used to determine which calendar years were recorded on the scale, thus leading to a correct age determination by simply knowing the date of capture.

The temporal signature technique is the matching of an individual's partial "environmental" growth history to characteristic increment patterns found in an "environmental" growth history defined for that species in a particular environment. The environmental growth component is the year-to-year expression of growth regardless of age and, thus, may be called a "year effect" (Weisberg 1993). A linear model, conceptualized as

(1) Expected growth increment = age effect + year effect + age × year interaction,

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TABLE 1. Number of fish, by scale-age and era, used to create the master chronology and assess the temporal signature technique. Only fish with a scale-age between four and eight were used to assess the temporal signature technique.

| | Create the master chronology | | | Assess the temporal signature technique | | |
|---|---|---|---|---|---|---|
| Scale-age | 1940s | 1960s | 1980s | 1940s | 1960s | 1980s |
| 2 | 0 | 0 | 157 | | | |
| 3 | 0 | 19 | 569 | | | |
| 4 | 8 | 39 | 158 | 0 | 12 | 10 |
| 5 | 13 | 43 | 5 | 9 | 15 | 2 |
| 6 | 13 | 37 | 2 | 10 | 13 | 0 |
| 7 | 4 | 31 | 1 | 7 | 6 | 0 |
| 8 | 6 | 8 | 8 | 6 | 0 | 2 |
| Total | 44 | 177 | 900 | 32 | 46 | 14 |

can be used to estimate the separate effects and coefficients (Weisberg 1993). A master chronology is the series of year coefficients estimated from a sample of known-age or reliably aged fish from a particular environment. This is analogous to the "standardization" used by dendroclimatologists to remove nonclimatic signals (e.g., age) in a tree growth-ring series (Graybill 1982). For an individual fish of unknown age, the environmental component of the recorded portion of the fish's growth history (i.e., observed increments minus estimated age coefficients) can be compared with the master chronology to determine which calendar years were recorded on the scale. For example, the eight increments recorded on the 20-yr-old fish, corrected for age by subtracting the corresponding age coefficients (1–8) from the observed increments, can be compared with every possible starting point (year) on the master chronology. The best estimate of the individual's membership in a year-class is the initial year of the 8-yr segment on the master chronology for which the age-corrected values best match. The age of the individual is then estimated as the difference between the date of capture and the estimated year-class.

This paper demonstrates the application of the temporal signature technique for assigning age to walleye (*Stizostedion vitreum*) from Red Lakes, Minnesota. It illustrates the strengths and limitations of the technique for assigning ages to a species whose older members are difficult or impossible to age by scale inspection. The shortcomings that are discussed for this data set are no less difficult than those commonly encountered in fisheries assessments. Thus, this example provides a realistic examination of the validity of this new technique.

## Methods

A master chronology for Red Lakes walleye was developed from fish collected in 89-mm (stretch measure) gill nets (Shroyer 1991; Table 1). Scales from these fish were pressed on acetate slides for viewing on a microfiche reader. An annulus, defined as the exact circulus which "cut-over" previous circuli (Shroyer 1991), was located with the aid of general criteria (Jearld 1983). Fish assigned an age older than 8 were not used in the development of the master chronology (Table 1) because scales are known to underestimate the age of older walleye (Belanger and Hogler 1982; Erickson 1983) and, on older fish, annuli near the scale margin could not be unambiguously located and measured, a requirement for applying the technique. Annual growth increments were measured with the aid of a digitizer tablet along a transect drawn from the focus to the anterior scale margin between the two centermost radii (Jearld 1983). All model fitting was performed with Weisberg's (1993) special purpose program assuming an identity, rather than a general, correlation matrix.

Model (1) was fit to the annual increment data from these samples. The estimated interaction effect was highly significant and its mean square was 1.0% of the age and 13.3% of the year mean square. A significant interaction indicates that each year-class, rather than the stock, has its own characteristic chronology. If a significant interaction occurs, then the temporal signature technique could be implemented by estimating a master chronology for each year-class rather than for the stock. However, the analysis is simpler and more intuitive if the age × year interaction explains little of the variation and the main effects can be interpreted directly. Weisberg (1993) described several techniques for diagnosing the cause of and effectively reducing a high age × year interaction. We reduced the age × year interaction by fitting a $\log_e$-transformed version of the conceptual model (1), namely

$$(2)\quad \log_e(\text{expected growth increment}) = \log_e(\text{age effect}) + \log_e(\text{year effect}) + \log_e(\text{age} \times \text{year interaction})$$

(Weisberg 1993), and excluding the first increment. The first increment may have contributed substantially to the interaction effect because growth of young-of-the-year walleye is poorly correlated with growth of older walleye in Red Lakes (Shroyer 1991).

Coefficients for ages 2–8 and the year coefficients for three noncontiguous periods 1942–49, 1961–72, and 1980–87, referred to as the eras of the 1940s, 1960s, and 1980s, were estimated by fitting (2) to all increments (except the first) from all fish. The interaction is significant but explains little of the variation relative to the two main effects (i.e., only 6.1% of the age and 0.4% of the year mean square; Table 2) and can be ignored (Weisberg 1993). Thus, the age and year coefficients are interpreted directly and, for use in later analyses, are put into a single vector, $A$, with $m$ age coefficients (=7 in this case) in the first $m$ rows and $k$ year coefficients (=28) in the next $k$ rows. Each matrix and subscript used in the analysis is briefly described in Table 3.

The effectiveness of the temporal signature technique was tested with a sample independent of that used to create the master chronology (Tables 1 and 4). These fish were also collected with 89-mm gill nets but were sampled from only those fish thought to be from a year-class, $c$ (based on scale-assigned age), represented in the master chronology. Criteria for annulus detection and measurement were the same as those used to create the master chronology. For these samples, let $x_{daj}$ be the vector of $a$ incremental measurements ($\leq m$) from observed annuli (first increment excluded) on scales from the $j$th captured fish in year $d$.

A simple error sum of squares was used to measure the concordance of a vector of $f$ measurable increments ($\leq a$) with the master chronology beginning at year $c + 1$ (the addition of 1 is necessary because the first increment was ignored). This measure, called concordance sum of squares (CSS), is expressed in matrix notation (similar to equation 2.19 of Weisberg (1985)) as

$$(3)\qquad \mathrm{CSS}_{cfj} = [(y_{dfj} - D_{cf}A)^T(y_{dfj} - D_{cf}A)]$$

where $A$ is the vector of age and year coefficients described previously, $y_{dfj}$ is a vector of $m$ components with the first $f$ rows

TABLE 2. Analysis of variance table for (2) fit to scale increments 2–8 from walleye used to create the master chronology.

| Source | df | SS | MS | $F$ | $p$ |
|---|---|---|---|---|---|
| Age | 6 | 444.017 | 74.003 | 1246.470 | <0.000001 |
| Year | 27 | 134.085 | 4.966 | 83.647 | <0.000001 |
| Interaction | 88 | 26.648 | 0.303 | 5.100 | <0.000001 |
| Pure error | 2805 | 160.595 | 0.060 | | |

TABLE 3. List and definition of all matrices and subscripts used in the temporal signature analysis.

*Matrices*

| | |
|---|---|
| $A$ | age and year coefficients from fit of (2) to all fish used to create the master chronology, $[(m + k) \times 1]$ |
| $x_{daj}$ | measurements of observed annual increments for a fish, $[a \times 1]$ |
| $y_{dfj}$ | $\log_e$-transformed measurements of the subset of annual increments to be compared with the master chronology $[m \times 1]$ |
| $D_{cf}$ | design matrix to identify which age and year coefficients of $A$ that $y_{dfj}$ will be compared with, $[m \times (m + k)]$ |

*Subscripts for matrices*

| | |
|---|---|
| $m$ | number of age coefficients estimated during fit of (2) |
| $k$ | number of year coefficients in master chronology |
| $a$ | number of observed annual increments on a fish (excluding the first), $\leq m$ |
| $j$ | unique fish identifier |
| $d$ | capture year of a fish |
| $f$ | number of observed annual increments to compare with master chronology, $\leq a$ |
| $c$ | a year on the master chronology |
| $z$ | presumed maximum age of the fish |

corresponding to the first $f$ values of the $\log_e$-transformed $x_{daj}$ and zeroes in the remaining $(m–f)$ rows, and $D_{cf}$ is an $m \times (m + k)$ design matrix of all zeroes with the exception of $f$ ones on the diagonal beginning in the upper left corner and another $f$ ones on the diagonal beginning in the first row of the $(c + 1)$st column (Table 5). Conceptually, the two diagonals of ones in $D_{cf}$ identify the $f$ age coefficients used to correct the observed growth increments for age and the $f$ years on the master chronology for fitting the age-corrected increments (Table 5). For each fish, $c$ was iterated for all years from 1941 to 1986 and $f$ was iterated from three (increments 2–4) to $a$ (all observed increments), except that comparisons with the master chronology were not made for starting years where fewer than $f$ increments would be compared. The minimum number of increments compared was chosen to be three ($f = 3$) because comparisons with $f = 2$ would be limited to only two unique patterns (i.e., increasing or decreasing). Most applications of the temporal signature technique will not test individual chronologies with $f < a$. However, we iterated $f < a$ to examine the sensitivity of the method in situations where only a small number of increments may be available. With these iterations, $\mathrm{CSS}_{cfj}$ was determined for each fish, all possible year-classes represented in the sample, and all combinations of successive increments beginning with the second (i.e., from increments 2–4 to 2–$a$). The best predicted year-class, $c$, for fish $j$ using $f$ increments is the year-class associated with the lowest $\mathrm{CSS}_{cfj}$. The best predicted age is the difference between the capture year ($d$) and the best predicted year-class, $c$.

The procedure just described is the analytical equivalent of "sliding" the age-corrected increment pattern of a specimen along the master chronology until a "best fit" is observed. The initial year of the best-matched interval is then taken to be the year-class of origin for that fish. This procedure closely resembles "cross-dating," the matching of tree-ring patterns in dendrochronology studies (Pilcher 1990). Our method of measuring "best fit" (i.e., CSS) is related to the correlation coefficient often used when cross-dating tree rings (Pilcher 1990) and the "bin sorter" algorithms of some neural network applications (Caudill 1993).

TABLE 4. Total number of comparisons used to assess the temporal signature technique arranged by scale-age and the number of the last increment used in the comparison with the master chronology.

| | Number of last increment used | | | | | |
|---|---|---|---|---|---|---|
| Scale-age | 8 | 7 | 6 | 5 | 4 | Total |
| 4 | | | | | 22 | 22 |
| 5 | | | | 16 | 26 | 42 |
| 6 | | | 15 | 17 | 23 | 55 |
| 7 | | 6 | 9 | 10 | 13 | 38 |
| 8 | 3 | 5 | 5 | 5 | 8 | 26 |
| Total | 3 | 11 | 29 | 48 | 92 | 183 |

The percentage agreement between scale-assigned age (scale-age) and temporal signature-assigned age (signature-age) was used as a measure of the effectiveness of the new technique to assign age. However, this measure of effectiveness is conservative because a fish's growth history is compared with all year classes represented in the master chronology. Any real application of this technique would necessarily restrict com-

TABLE 5. Two examples of design matrices (blocks of zeros and ones) for comparing growth histories with the master chronology. In example A, increments 2–5 are compared with the master chronology beginning in 1944 ($D_{1943,4}$). In example B, increments 2–7 are compared with the master chronology beginning in 1967 ($D_{1966,6}$).

| | Scale-age | | | | | | | Growth year | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Scale-age | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 1942 | | | | | | | | 1961 | | | | | | | | | | | | 1979 | | | | | | | |
| | | | | | | | | | | | | | | | | *Example A* | | | | | | | | | | | | | | | | | | | |
| 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | | | | | | | | | | *Example B* | | | | | | | | | | | | | | | | | | | |
| 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

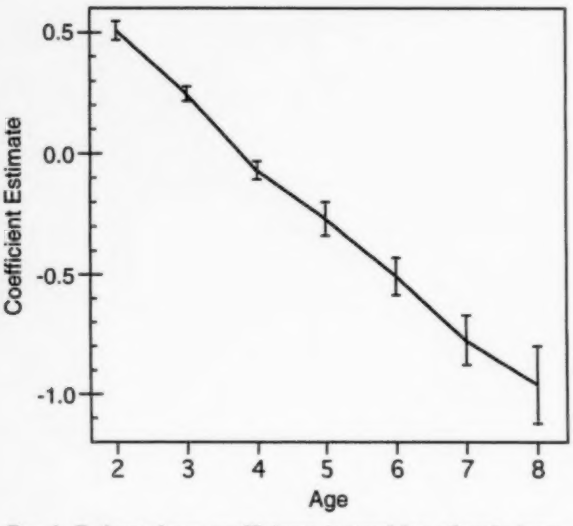

FIG. 1. Estimated age coefficients (natural log of scale increment with Bonferroni-corrected 95% confidence intervals) from (2) fit to increments 2–8 (from Shroyer's (1991) table 12).

parisons only to those years on the master chronology prior to the year of capture. To simulate this condition without unnecessarily inflating percentage agreement by reducing the number of comparisons, we restricted the analysis to those years on the master chronology that were within 5 yr of *d*–*a* (i.e., the number of observed increments before the capture year).

## Results

Scale increments of Red Lakes walleye differed by age and by year corrected for age (Table 2). Walleye growth increments declined linearly with increasing age (Fig. 1). Growth of Red Lakes walleye was poorer in every year between 1942 and 1986 relative to 1987, the last growth year represented in the data (Fig. 2). Year coefficients fluctuated greatly in the 1940s and 1960s, but remained constant or increased in the 1980s. Standard errors were larger for the growth years at the beginning of each of the three periods (Fig. 2) because growth in these years was represented in only a few fish (Table 1).

Scale-age equaled one of the three most likely signature-ages in 67–77% of the comparisons using all observed increments, regardless of age (Table 6). For fish younger than age 8, scale-age equaled the most likely signature-age in 40–67% of the comparisons when all observed increments were used. For age-8 fish, scale-age never equaled the most likely signature-age but did equal the second or third most likely signature-age in 67% of the comparisons when all observed increments were used. Within each scale-age-class, percent agreement generally declined as a decreasing number of observed increments were compared with the master chronology (Table 6). Restricting the analysis to those year-classes within 5 yr of the year-class assigned by the scale-age, agreement between scale-age and the most likely signature-age ranged from 33 to 69% when all observed increments were used (Table 7).

As an example of the temporal signature technique, a walleye captured in 1951 and assigned a scale-age of 8 (i.e., presumably from the 1943 year-class) was compared with the master chronology with $f = 4$ and $f = 6$. The year-class with the lowest CSS was 1966 for $f = 4$ and 1943 for $f = 6$ (Fig. 3). If the $f = 4$ result is disqualified by the year-class restriction, then the year-class with the lowest CSS was 1943.

## Discussion

This example demonstrates that the temporal signature technique can be used to accurately assign age to a fish from the unambiguously recorded (i.e., permanent, with distinct annuli)

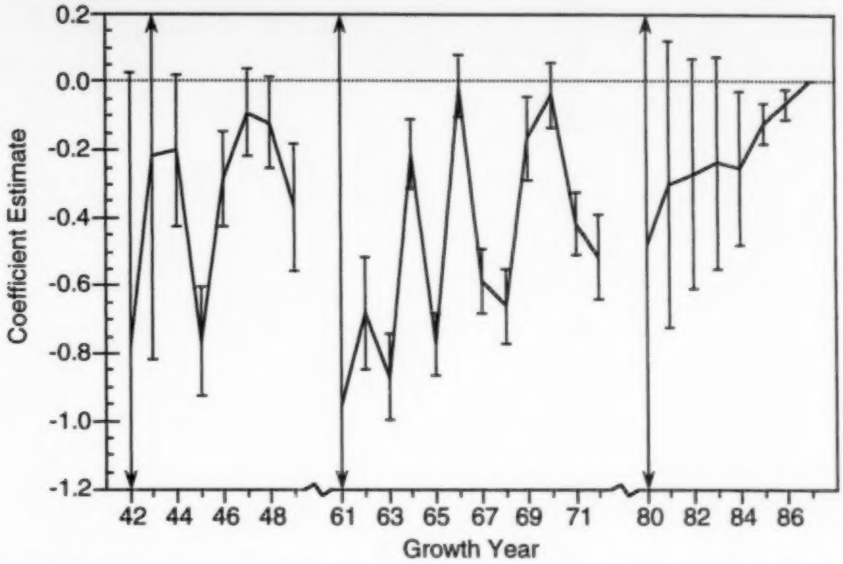

FIG. 2. Estimated year coefficients (natural log of scale increment with Bonferroni-corrected 95% confidence intervals) from (2) fit to increments 2–8 (from Shroyer's (1991) table 13). All year coefficients are relative to 1987 which was set equal to zero. This series of coefficients is considered the master chronology for Red Lakes walleye for the years represented.

inner portion of its scales. Signature-age agreed with scale-age most often when all observed increments were used. Agreement was also good when some of the observed increments were not used. This latter result reveals two important features of the temporal signature technique. First, the temporal signature technique can be used to assign age to scales with marginally indistinct or incompletely recorded growth histories. Second, the portion of the growth history that is most easily interpreted on scales (i.e., a small number of the innermost increments) can be used to assign age.

In most situations, there will be a minimum number of unambiguously observed growth increments on the inner portion of a scale that are required to accurately determine the correct age. In this example, there was little difference when only three rather than more increments were compared with the master chronology. However, disagreement between scale-age and signature-age increased when some observed increments were not used, although scale-age equaled the signature-age with the second or third lowest CSS in many of these misclassifications. Thus, all increments that can be unambiguously observed should be used, but more importantly, the temporal signature technique may be sufficient when the number of unambiguously observed increments is small, possibly as few as three.

TABLE 6. Percentage of comparisons where the scale-age equaled the signature-age with the lowest (rank =1) or one of the three lowest (rank ≤ 3) concordance sum of squares (CSS). All observed scale increments were used in comparisons where the last increment used equaled the scale-assigned age.

| | Last increment ($f$ + 1) compared with the master chronology | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Scale-age rank = 1 | | | | | Scale-age rank ≤ 3 | | | | |
| Scale-age | 8 | 7 | 6 | 5 | 4 | 8 | 7 | 6 | 5 | 4 |
| 4 | | | | | 59 | | | | | 77 |
| 5 | | | | 44 | 27 | | | | 69 | 54 |
| 6 | | | 40 | 47 | 35 | | | 73 | 76 | 61 |
| 7 | | 67 | 33 | 20 | 8 | | 67 | 44 | 60 | 39 |
| 8 | 0 | 0 | 20 | 20 | 0 | 67 | 40 | 60 | 60 | 50 |

The temporal signature technique will more accurately assign ages if the growth histories of adjacent year-classes are markedly different from each other. In long master chronologies, the growth history of a given year-class will likely closely resemble some other year-class or year-classes. However, if the year-classes with similar patterns are several years apart, then other information (e.g., capture year or size at capture) may be used to distinguish between the two. The distinctiveness of a given year-class relative to adjacent year-classes can be determined by comparing the predicted growth history of a year-class with the predicted growth history of all other year-classes and then determining the rank of the fit to adjacent year-classes. In Red Lakes walleye, year-classes in the 1940s and 1960s were relatively distinct (median rank of adjacent year-classes 1 and 2 yr removed was 13 and 9) whereas year-classes in the 1980s were not distinct (median rank of adjacent year-classes 1 and 2 yr removed was 1.5 and 2). Distinct patterns in a master chronology will result in better age assignments because comparisons will rely more on relative pattern matching than on the absolute magnitude of the increments. Thus, the ability of this technique to determine the correct age of a fish will be influenced by the variability of external factors (e.g., climate and intra- or interspecific densities) and the sensitivity of a species' response to those factors.

Restricting the comparisons to only plausible year-classes on the master chronology would increase the accuracy of this technique. Plausible year-classes can be defined as the set of all year classes between $f$ and $z$ (maximum age of the species) years prior to the capture year. The restriction to plausible

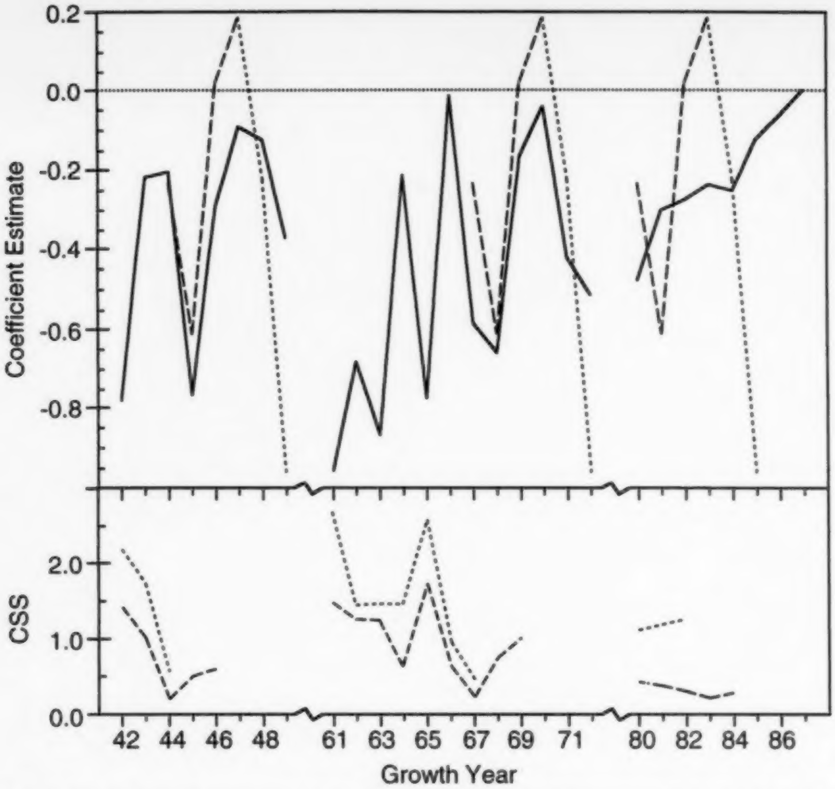

FIG. 3. Comparison of increments 2–5 ($f = 4$; dashed line) and increments 2–7 ($f = 6$; dashed and dotted line) of a Red Lakes walleye captured in 1951 and assigned an age of 8 (1943 year-class) with the master chronology (solid line) at three possible initial years (upper panel). The concordance sum of squared deviations (CSS) for all comparisons is shown in the lower panel. The growth year with the lowest CSS was 1967 (1966 year-class) for $f = 4$ and 1944 (1943 year-class) for $f = 6$.

TABLE 7. Percentage of all comparisons for which scale-age differed from signature-age by −1, 0, and 1 yr when year-classes more than 5 yr removed from the year-class determined by the scale-age were exempted from the analysis. Corresponding cells in each subtable do not sum to 100% because larger differences are not shown.

| | Last increment ($f + 1$) compared with the master chronology | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Scale − signature = −1 | | | | | Scale = signature | | | | | Scale − signature = 1 | | | | |
| Scale-age | 8 | 7 | 6 | 5 | 4 | 8 | 7 | 6 | 5 | 4 | 8 | 7 | 6 | 5 | 4 |
| 4 | | | | | 0 | | | | | 68 | | | | | 5 |
| 5 | | | | 0 | 0 | | | | 69 | 42 | | | | 0 | 12 |
| 6 | | | 0 | 0 | 9 | | | 60 | 59 | 43 | | | 7 | 6 | 4 |
| 7 | | 17 | 11 | 20 | 23 | | 67 | 67 | 60 | 46 | | 0 | 11 | 10 | 8 |
| 8 | 0 | 0 | 20 | 20 | 13 | 33 | 20 | 20 | 20 | 38 | 0 | 0 | 0 | 0 | 0 |

year-classes was not implemented in this analysis because the partial growth history of some samples would have been compared with only a small number of year-classes due to discontinuities in the master chronology. However, the more conservative restriction implemented in this example (i.e., restricting comparisons to years on the master chronology within 5 yr of *d–a*) resulted in an increase in the percent agreement between the two age assignment methods.

The temporal signature technique will be enhanced by the development of other measures of fit to the master chronology. The new measures may be equally simple, such as measuring the correlation between an individual's growth history and the master chronology, or more complex, an an application of neural network algorithms (Caudill 1993) or development of a Bayesian approach. In a Bayesian approach, a likelihood function constructed from the concordance of a sample with the master chronology could be used to modify a prior distribution of possible ages that was formed from a scale reader's interpretation of all features on the scale and information about the sample (e.g., length, sex, and location). Probabilities of age obtained from the updated distribution (i.e., posterior distribution) would provide an estimate of confidence in the age determined with this method. The probabilities of age in the posterior distribution could be used to assign an age to the fish (i.e., the age with the highest posterior probability) or eliminate the fish from further consideration (i.e., all posterior probabilities are equally low).

The master chronology would best be determined from known-age fish, but "reliably ageable" fish can provide a sound basis for a master chronology. Inclusion of fish in the master chronology could simply be limited to those fish that are younger than the age where age assignment becomes uncertain. The age where uncertainty begins for a particular species may be identified by analysts familiar with age determination from scales, by experience with other calcified structures (e.g., Erickson 1983), or from scale validation results.

In our example, two 1988 fish aged from scales as 8-yr-olds were assigned to the 1979 year-class by temporal signatures. This was true for all of the increment sets used to estimate temporal signature-age ($f = 3, 4, 5, 6,$ and 7) for these fish. An operculum from one of these fish yielded an age assignment of 9, which implies that the scale-age determination was erroneous. The possibility remains that our master chronology included some older fish that were misaged from scales. In spite of whatever errors of this kind are currently included within our master chronology, the scale-ages from the test sample are substantially in agreement with the signature-ages. It might be reasonable to conclude from the discontinuity evident in Table 6 for age-8 fish that age 7 is the oldest scale-age determination that should be allowed to contribute to the master chronology.

The Red Lakes walleye example represents, in our opinion, the coarsest level of resolution that might be expected from the temporal signature approach to age estimation. Discontinuities in the master chronology, simplistic analytical techniques (e.g., CSS), and purposely conservative analytical decisions (e.g., allowing an individual's growth history to be fitted to year-classes subsequent to the capture year) led to results that underestimate the ability of this technique to assign accurate ages to fish from partial growth histories. Continuous master chronologies, development of new techniques for judging the fit of a growth history to the master chronology, restrictions of plausible year-classes, and, possibly, the use of within-year patterns, especially during the first year of life, will greatly enhance the accuracy and applicability of this technique.

We believe that the temporal signature approach is superior to age determination based solely upon visual examination of scale growth. No analyst, no matter how experienced, can judge the age of a fish exhibiting only a portion of its growth history on its bony structures. Our approach removes that subjectivity and provides a method which could be automated. In addition, one of the most appealing features of this method is the assignment of probabilities to membership in specific age-classes.

The temporal signature technique will increase our understanding of fish populations in two ways. First, samples that were previously ignored because of age assignment difficulties (Carlander 1987) can now be exploited to more accurately represent and understand past fish populations. Historical collections of fish scales should, therefore, be protected because there is now a method to utilize these samples. Second, master chronologies that extend to the present can be used to assign age to scale samples from contemporary collections. Because scales are inexpensive and nondestructive structures to collect (Jearld 1983), we believe that this analytical technology will enhance the accuracy and precision of future stock assessment.

## Acknowledgments

This paper is respectfully dedicated to the memory of J.B. Smith and F.E.J. Fry for their insight into the importance and ambiguity of age interpretation from fish scales. We thank D.L. Pereira for scale-assigned ages on some of the samples used to assess the technique and D. Conner and the Red Lakes Band of Chippewa for access to recent collections of Red Lakes scale samples. This manuscript benefited from critical review by L.A. Bergquist, A.T. McClure, D.L. Pereira, B.C. Vondracek, S. Weisberg, and three anonymous referees. This paper is published as paper No. 20 972 of the scientific series of the Minnesota Agricultural Experiment Station based on research conducted under Project 77.

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# Effects of Intermittent Chloramine-T Exposure on Growth, Serum Biochemistry, and Fin Condition of Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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Powell, M.D., D.J. Speare, and N. MacNair. 1994. Effects of intermittent chloramine-T exposure on growth, serum biochemistry, and fin condition of juvenile rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 51: 1728–1736.

Chloramine-T is a commonly used therapeutic agent for the treatment of bacterial gill and skin diseases in the intensive aquaculture of salmonids; however, little is known about its effects on healthy fish. Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed weekly to 5, 10, and 20 mg active chloramine-T ingredient·$L^{-1}$ in a 20-tank replicate flow-through facility. Control fish were treated with tank water only. Growth parameters and haematocrits were taken twice weekly and serum biochemistry was measured on termination of the experiment. Absolute growth rates were suppressed but only statistically significantly in fish treated with 20 mg chloramine-T·$L^{-1}$ compared with controls. Fish exposed to 10 and 20 mg chloramine-T·$L^{-1}$ showed significant predisposition to an erosive dermatitis of the caudal fin which appeared to be caused by opportunistic pathogens of the genus *Pseudomonas* spp. and *Flavobacter* spp. Haematocrits decreased in controls and all treatments. A significant concentration-dependent decrease in plasma sodium and chloride was observed. Plasma glucose levels did not vary and were similar to those reported for unstressed trout. We recommend that care be taken when using chloramine-T prophylactically and that concentrations of less than 10 mg·$L^{-1}$ be used.

La chloramine-T est un agent thérapeutique couramment utilisé pour le traitement des maladies bactériennes des branchies et de la peau dans l'aquaculture intensive des salmonidés; toutefois, on ne connaît pas très bien ses effets sur les poissons en santé. Des truites arc-en-ciel juvéniles ont été exposées une fois par semaine à 5, 10 et 20 mg de matière active de chloramine-T·$L^{-1}$ dans une installation avec renouvellement continu où 20 bassins ont été utilisés en parallèle. Les poissons témoins ont été traités avec de l'eau du bassin uniquement. Les paramètres de la croissance et l'hématocrite ont été mesurés deux fois par semaine et les paramètres biochimiques sériques ont été mesurés à la fin de l'expérience. La valeur absolue du taux de croissance a diminué, mais cette diminution n'était significative que chez les poissons traités à raison de 20 mg de chloramine-T·$L^{-1}$, comparativement aux témoins. Les poissons exposés à la chloramine-T à raison de 10 et de 20 mg·$L^{-1}$ ont été significativement plus prédisposés à une dermatite érosive de la nageoire caudale, causée apparemment par des agents pathogènes opportunistes appartenant aux genres *Pseudomonas* et *Flavobacter*. L'hématocrite a diminué chez les témoins et tous les groupes de traitement. On a observé une diminution significative des teneurs en sodium et en chlore du plasma, liée à la concentration de la chloramine-T. La concentration de glucose dans le plasma n'a pas varié et était semblable aux concentrations signalées pour des truites non soumises à un stress. Nous recommandons d'être prudent en utilisant la chloramine-T à titre prophylactique et d'utiliser des concentrations inférieures à 10 mg·$L^{-1}$.

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Topical infections involving the colonization of gills and skin with a range of pathogens are a common problem in the high-intensity rearing of fish including salmonids. Accordingly, treatments for these diseases are commonly used although the effects on the host are minimally understood. Chloramine-T is a formulation commonly used by aquaculturists worldwide. In a survey of 62 Ontario trout farms, a province in which gill diseases, particularly bacterial gill disease (BGD), are very common (Daoust and Ferguson 1983; Speare and Ferguson 1989), chloramine-T bath treatments are the primary treatments of choice used on 66% of farms (Thorburn and Moccia 1993). Thorburn and Moccia (1993) found that chloramine-T was used both as a therapeutic agent and as a prophylactic-type of preventative treatment (in 36% of treatment regimes, 13% of farmers using chloramine-T as a prophylactic treatment only). In a similar study of trout hatcheries in Virginia, U.S.A., 68% of hatcheries used chloramine-T (Smith et al. 1993). A survey of finfish submissions for postmortem examination to the Atlantic Veterinary College (AVC) Fish Health Unit (FHU) between January 1988 and June 1991 (AVC-FHU diagnostic data, D.J. Speare, Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, P.E.I., Canada) revealed that 48% of submitted clinical cases were diagnosed with gill or skin diseases. The primary drug of choice to treat bacterial skin and gill diseases in Prince Edward Island is chloramine-T (32% of cases submitted had been treated with chloramine-T prior to submission to the AVC-FHU). These submissions were prompted by a marked increase in mortality and failure of therapeutic treatment. Although more specific data are not available for other regions in North America, the available data suggest that chloramine-T is widely and commonly used in North American fish hatcheries.

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Little is known about the mechanisms of therapeutic action of chloramine-T on fish. Acute chloramine-T (*n*-sodium-*n*-chloro-para-toluenesulfonamide) toxicity has been addressed for rainbow trout (*Oncorhynchus mykiss*) (Bills et al. 1988). It has been shown that chloramine-T toxicity is temperature as well as pH and water hardness dependent (Bills et al. 1988). In rainbow trout the water temperature at the time of the exposure affected the time of toxic response rather than the ultimate toxicity of chloramine-T. Chloramine-T was found to be slightly more toxic in soft water compared with hard water. However, there was a significant increase in toxicity with a decrease in pH, the drug being six times more toxic at pH 6.5 than at 9.5. Durations of exposure of less than 3 h under any of the tested environmental conditions resulted in median lethal concentrations ($LC_{50}$) above the recommended treatment dosages for bacterial gill disease in trout (Bills et al. 1988). Concentrations ranging from 6.5 to 10.0 mg chloramine-T$\cdot L^{-1}$ have been demonstrated as effective therapy for the treatment of BGD (From 1980; Speare and Ferguson 1989; Bullock et al. 1991). It is not known whether chloramine-T acts through a bactericidal action or due to enhanced physiologic clearance of the colonised biofilm overlying the host epithelium.

The mechanism for chloramine-T toxicity is not fully understood, but it is known to release chlorine (Booth and McDonald 1988). Chlorine is highly reactive with ammonia-containing compounds, resulting in the pH-sensitive formation of mono- and dichloramines (Brooks and Bartos 1984). The unreacted (free) chlorine and mono- and dichloramines are highly toxic to fish. Rainbow trout are particularly sensitive to chlorine and chloramines, with a 30-min $LC_{50}$ at 20°C of monochloramine dichloramine, and hypochlorous acid of 1.35, 0.57, and 0.46 mg$\cdot L^{-1}$, respectively (Brooks and Bartos 1984). The toxicity of chlorine is temperature dependent. At 10 and 15°C the 30-min $LC_{50}$ of residual chlorine (of which 75 and 50%, respectively, was free chlorine) for rainbow trout has been demonstrated at 0.99 and 0.94 mg$\cdot L^{-1}$, respectively (Brooks and Seegert 1977). However, the toxic effects of chlorine and ammonia may be confounded by elevated ammonia concentrations working synergistically, increasing the apparent net toxic effect (Mitchell and Cech 1983).

The object of this investigation was to experimentally examine growth and selected blood indices of healthy rainbow trout following repeated intermittent static exposure to chloramine-T. It should be stressed, however, that the results of this study are preliminary and further investigation is continuing to elaborate the underlying physiological mechanisms of toxicity.

## Materials and Methods

### Fish Holding

A system of 20 replicate tanks modified from the system described by Bills et al. (1988) and recommended by the ASTM Committee E-35 on Pesticides (1980) and according to the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) was used for this investigation. This design allowed the random designation of five tanks for each concentration of chloramine-T to be tested. All the tanks were supplied with a common source of well water at the AVC-FHU.

A total of 400 sibling laboratory-hatched 0+ rainbow trout juveniles of mean weight 16.5 g (±SD 6.0 g) were supplied by Dr. C.E. Johnston (Department of Biology, University of Prince Edward Island). Examination of an arbitrarily selected subsample of the fish population showed the fish to be free from branchial pathology. Fish were randomly allocated to one of twenty 12-L experimental flow-through polyethelene tanks (20 fish per tank) at a mean stocking density of 2.77 kg$\cdot m^{-3}$ (±SD 0.10 kg$\cdot m^{-3}$). The mean tank water inflow rate was measured at 0.83 L$\cdot min^{-1}$ (±SD 0.17 L$\cdot min^{-1}$). Supplemental aeration of the tank water was provided by individual air stones in each experimental tank. Fish were maintained for a habituation period of 3 wk prior to the start of experimentation and fed a commercially prepared 2.5-mm pelleted feed (Corey, Fundy Choice) daily ad libitum. Uneaten feed and faecal wastes were removed daily by siphoning. During the habituation period and in subsequent experiments, water temperature was maintained at 11.0 ± 0.5°C, and the fish were exposed to a 12 h light : 12 h dark photoperiod. During the habituation period the water inflow to one of the control tanks was interrupted resulting in a large mortality of the fish. This tank was withdrawn from experiments.

### Experimental Protocol

Following the habituation period, fish were fed once daily at a rate of 2% of the mean tank biomass with the same commercial pelleted feed preparation as used in the habituation period per day for 22 d. This amount was corrected weekly for each tank as fish were removed during the experiment. Uneaten feed and faecal solids were removed daily by siphoning. Feeding was suspended 24 h prior to chloramine-T exposure in all tanks (including controls) and solids removed by siphoning. Fish were exposed to 5, 10, and 20 mg chloramine-T (active ingredient)$\cdot L^{-1}$ in a static bath. To achieve this, the water inflow to each tank (including control tanks) was stopped and chloramine-T (Syndel Laboratories, Vancouver, B.C., Canada) (predissolved in 25 mL of tank water) was added to each test tank. Controls were exposed to 25 mL of tank water only. Fish were maintained for 1 h before the water inflow was restored. Exposures were repeated twice weekly on consecutive days (Monday and Tuesday) for 4 wk.

### Sampling Protocol and Data Analysis

Fish were sampled immediately prior to and immediately following each week's exposure. It was thus possible to assess the effects of treatment on blood haematocrit and make two measurements of growth parameters. One fish was removed from each tank ($n = 5$ for experimental tanks, $n = 4$ for control tanks) and rapidly euthanised by spinal severance. Blood was collected with a heparinised haematocrit tube from the severed dorsal aorta and centrifuged at 3500 rpm for 5 min. The carcass was weighed, measured, and examined for signs of skin damage or infection.

Condition factor (*K*-factor) using wet weight and fork length measurements was calculated according to the formula (Busacker et al. 1990)

TABLE 1. Ionic composition of the common inflowing water to the replicate experimental tank units.

| Cation | Concentration ($mg \cdot L^{-1}$) | Anion | Concentration ($mg \cdot L^{-1}$) |
|---|---|---|---|
| Cadmium | <0.005 | Chloride | 80.000 |
| Calcium | 45.860 | Nitrate | 2.700 |
| Chromium | <0.050 | Sulphate | 14.580 |
| Copper | <0.020 | pH | 7.4 |
| Iron[a] | <0.100 | | |
| Iron[b] | <0.100 | | |
| Lead | <0.050 | | |
| Magnesium | 22.820 | | |
| Manganese | <0.020 | | |
| Nickel | <0.050 | | |
| Phosphorus | 0.180 | | |
| Potassium | 2.160 | | |
| Sodium | 25.920 | | |

[a] Dissolved iron.

[b] Extractable (complexed) iron.

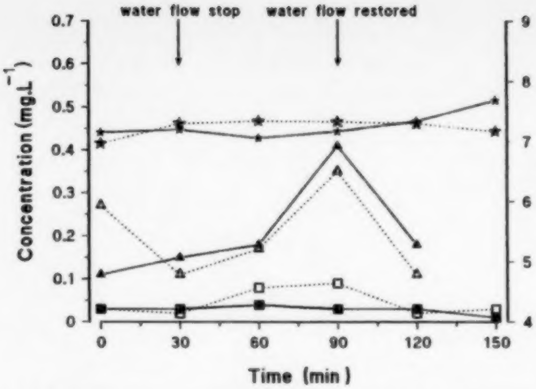

FIG. 1. Water chemistry profile during a 1-h static bath exposure of 20 rainbow trout fingerlings to 20 mg chloramine-T$\cdot L^{-1}$. Solid squares, free chlorine (control, 0 mg$\cdot L^{-1}$); open squares, free chlorine (20 mg$\cdot L^{-1}$); solid triangles, total $NH_3$-N (control 0 mg$\cdot L^{-1}$); open triangles, total $NH_3$-N (20 mg$\cdot L^{-1}$); solid stars, pH (control, 0 mg$\cdot L^{-1}$); open stars, pH (20 mg$\cdot L^{-1}$).

$$(1)\quad K = 100 \cdot \{\text{wet weight } (W)/\text{fork length}(L)^3\}.$$

Growth data were analysed using linear regression (Biosoft Inc. FigP graphics software (1990)), assuming any growth or change in condition of the fish over the 22-d period of the chloramine-T exposures to be linear (Busacker et al. 1990). A positive slope of the linear regression line was therefore indicative of the growth rate of the fish. Using the assumption that growth over the 22-d period of the experiment would be linear, the absolute growth rate could be calculated using the equation

$$(2)\quad (W_t - W_i)/t$$

where $W_t$ is the weight at time $t$, $W_i$ is the *initial* wet weight, and $t$ is the time frame of the experiment (Hopkins 1992). With linear regression analysis, this calculation is equivalent to the slope of the regression line (β value). The slope of the regression line would therefore be equivalent to the weight gain per day, the absolute growth rate (Hopkins 1992). Alternatively, if fork length is substituted for weight in equation 2, length gain per day can be derived. Statistically significant deviation of this slope from zero was indicative of significant growth. Regression line slopes were statistically compared using $t$-tests (Glantz 1987). A probability level of less than 0.05 was considered to be significant.

Haematocrits were calculated from blood samples taken from each fish and analysed by two-way analysis of variance. Blood plasma from samples taken from after the last two chloramine-T treatments was pooled and assayed for plasma sodium, potassium, chloride, calcium, glucose, and total protein concentration. Assays were determined with a Hitachi 911 automatic blood analyser using Boehringer Mannheim reagents. Mean plasma ion, glucose, and protein concentrations were compared for each concentration of chloramine-T using analysis of variance and multiple comparisons made with Bonferoni-corrected $t$-tests (Glantz 1987). Values of $p$ of 0.05 or less were considered to be significant.

The incidence of skin lesions was analysed using chi-square, and following assessment of the normality of the data, proportions of sampled fish at each dose with lesions were statistically compared using analysis of variance (Minitab™ Inc.). No transformations were required to meet the assumption of normal distribution of the data. Fish exhibiting skin lesions or skin damage were examined more closely and the tissue from the affected area fixed in 10% neutral buffered formalin and processed for histology. The lesion was cultured on cytophaga agar (CA) and blood agar (BA) plates and incubated at 22°C. The kidney was similarly cultured on BA for examination of systemic pathogens. Any resulting bacterial growth was identified with a combination of biochemical tests and nonfermentative test strips (API-NFT, BioMerieux Vitek Inc., Hazelwood, MO 63042, USA). Numbers of bacteria were quantified according to the amount of growth on the plate and determined as low, moderate, or high. Control fish were also examined and bacteriological smears and histological samples prepared as for fish exhibiting lesions.

## Water Chemistry

Samples of the inflowing well water were collected and analysed by the P.E.I. Department of the Environment laboratory at the beginning of the experiment (Table 1). In a separate trial after the last experimental exposure, a tank that had been exposed to 20 mg chloramine-T$\cdot L^{-1}$ and a control tank were used to determine oxygen saturation and total gas saturation profiles during a chloramine-T exposure. Oxygen and total gas saturation were measured using a Rustrack Ranger™ portable data logger with a YSI model 54 dissolved oxygen meter and a Nova Tech Designs Ltd. Tensinometer 300c, respectively. Similarly, concentrations of free chlorine were assessed at 30-min intervals during the exposure by DPD colorimetry as described in Franson (1978) using a DR/2000 spectrophotometer (Hach Company, Loveland, Colorado, U.S.A.). Total nitrogen-ammonia ($NH_3$-N) was also monitored at 30-min intervals during the exposure protocol using the salicylate method recommended in Walters (1989) using the Hach DR/2000 spectrophotometer.

The water supply used was quite hard (total hardness 208.89 mg$\cdot L^{-1}$) due to high concentrations of cations, particularly calcium and sodium (Table 1). The water also had a high alkalinity (136.00 mg$\cdot L^{-1}$) due primarily to high concentrations of chloride and sulphate (Table 1). The high hardness and alkalinity imparted a potentially high buffering capacity to the water flowing into the system. The water chem-

TABLE 2. Summary of growth indices from rainbow trout treated with chloramine-T following linear regression analysis of each parameter against time ($\beta$ = slope of regression line and equivalent to the absolute growth rate of the fish; $r$ = correlation coefficient).

| Growth parameter | Treatment | $\beta$ | $r$ | $t$-statistic | $p$ |
|---|---|---|---|---|---|
| Weight | Control | 0.469 | 0.778 | 2.774 | 0.039[a] |
| | 5 mg·L$^{-1}$ | 0.130 | 0.451 | 1.132 | 0.309 |
| | 10 mg·L$^{-1}$ | 0.160 | 0.392 | 0.952 | 0.385 |
| | 20 mg·L$^{-1}$ | −0.171 | −0.430 | −1.067 | 0.335[b] |
| Length | Control | 1.327 | 0.777 | 2.764 | 0.040[a] |
| | 5 mg·L$^{-1}$ | 0.465 | 0.697 | 2.162 | 0.083 |
| | 10 mg·L$^{-1}$ | 0.455 | 0.443 | 1.104 | 0.320 |
| | 20 mg·L$^{-1}$ | −0.171 | −0.339 | −0.806 | 0.457[b] |
| Weight/ length | Control | 0.026 | 0.769 | 2.690 | 0.043[a] |
| | 5 mg·L$^{-1}$ | 0.006 | 0.402 | 0.981 | 0.371 |
| | 10 mg·L$^{-1}$ | 0.009 | 0.346 | 0.825 | 0.447 |
| | 20 mg·L$^{-1}$ | −0.013 | −0.498 | −1.284 | 0.255[b] |
| $K$-factor | Control | −0.005 | −0.431 | −1.067 | 0.335 |
| | 5 mg·L$^{-1}$ | −0.003 | −0.510 | −1.325 | 0.242 |
| | 10 mg·L$^{-1}$ | −0.010 | −0.114 | −0.255 | 0.808 |
| | 20 mg·L$^{-1}$ | −0.004 | −0.350 | −0.834 | 0.442 |

[a] Slope significantly different from 0 at $p < 0.05$.

[b] Slope significantly different ($p < 0.05$) from control (0 mg chloramine-T·L$^{-1}$).

istry profiles of the tank of fish exposed to chloramine-T (20 mg·L$^{-1}$) and control tank (0 mg·L$^{-1}$) showed an elevation in total $NH_3$-N following cessation of water flow to the tanks (Fig. 1). The initial decrease in $NH_3$-N concentration in the tank treated with chloramine-T was probably due to increased swimming activity and opercular movements (respiration) which were observed and was probably a response to the introduction of oxygen probes into that tank for measurement of gas saturation during the treatment. Prior to the cessation of water flow and the addition of chloramine-T, the total ammonia levels had returned to background levels (Fig. 1). Addition of 20 mg chloramine-T·L$^{-1}$ to the tank resulted in an elevation in the concentration of free chlorine which rapidly subsided to background and control levels following return of water flow (Fig. 1). Nevertheless the concentration of free chlorine was somewhat lower than that reported by Bullock et al. (1991) who measured levels of up to 0.2 with 8.5 mg chloramine-T·L$^{-1}$. The reason for this discrepancy may be due to the high chlorine demand (caused by elevated $NH_3$-N levels) in our study, thus inducing the formation of mono- and dichloramines. There was little change in pH during the treatment period for either the tank treated with 20 mg chloramine-T·L$^{-1}$ or the control tank (Fig. 1). Minimum oxygen saturation during chloramine-T exposures was 82.75% and total dissolved gases was 96.25% saturation. During a separate monitoring of oxygen and total gas saturation of the control tank, minimum oxygen and total gas saturation were 87.50 and 97.00%, respectively.

## Results

### Observations on Fish Behaviour

Fish treated with 10 and 20 mg chloramine-T·L$^{-1}$ began crowding at the top of their respective tanks following the first treatment (day 3). These fish generally were dark in colour. Similarly during the chloramine-T exposure, those fish exposed to 10 and 20 mg·L$^{-1}$ showed increases in the amplitude and frequency of ventilatory movements. Such changes were not evident 24 h after the termination of the exposure. Fish exposed to 5 mg·L$^{-1}$ and control fish were evenly distributed throughout the tank, and although some increase in ventilation frequency was noticed, the fish appeared unaffected by the treatment. Some of the fish exposed to 5 mg chloramine-T·L$^{-1}$ began to take on a dark colouration following the third week of treatment (day 15). All fish appeared to be feeding normally. Faecal material was recovered from all tanks along with some uneaten pellets although the respective amounts were not weighed. There was no apparent difference in the amount of uneaten pellets between control and experimental tanks.

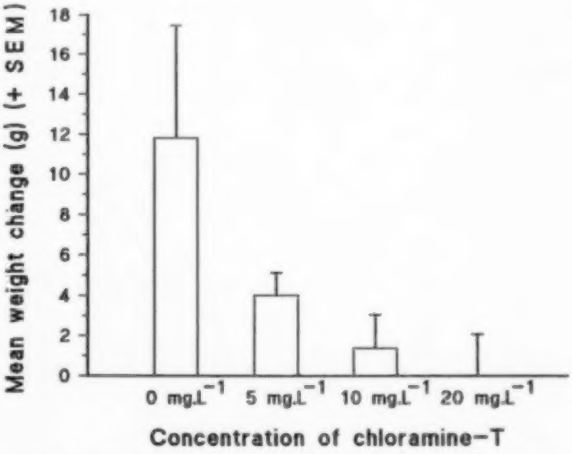

FIG. 2. Increase in mean wet weight (+SEM) over the 22-d experimental period for fish intermittently exposed to chloramine-T.

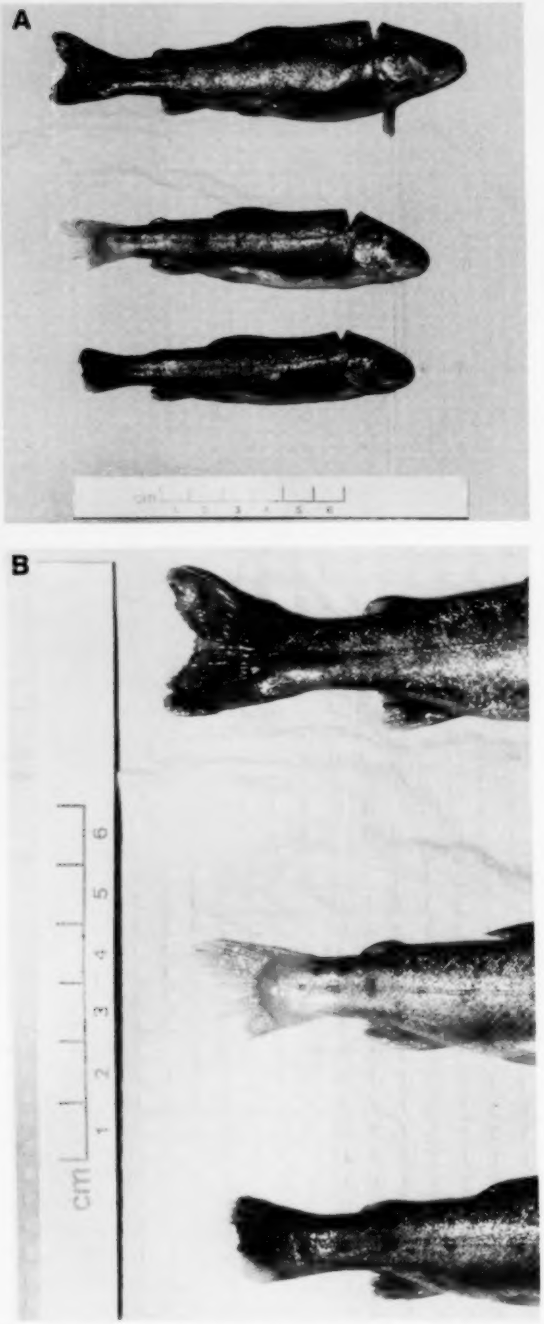

FIG. 3. (A) Difference in overall appearance and (B) erosion of the caudal fin of control fish (top) and fish intermittently exposed to 10 mg chloramine-T·L$^{-1}$ (middle) and 20 mg chloramine-T·L$^{-1}$ (bottom).

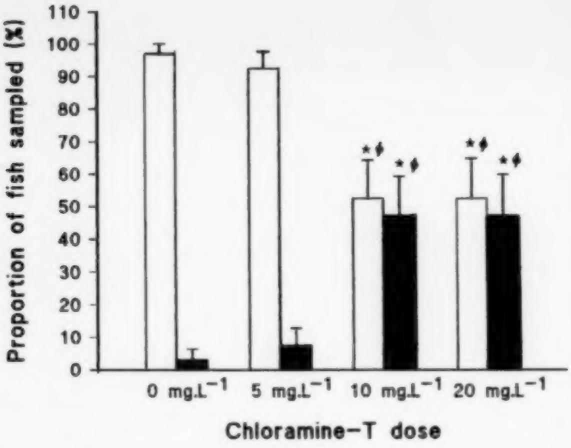

FIG. 4. Mean proportion of fish sampled (+SEM) with caudal fin erosion over a 4-wk period of treatment with chloramine-T. Open bars, proportion of apparently healthy fish with no erosion or necrosis; solid bars, proportion of fish with caudal fin erosion or necrosis; stars, values significantly different from controls; phi's, values significantly different from 5 mg·L$^{-1}$.

## Growth

Significant increases ($p < 0.05$) in weight and length parameters were seen in control fish but were not evident in fish treated with chloramine-T at any of the experimental doses (Table 2). Weight and length of fish exposed to 20 mg chloramine-T·L$^{-1}$ exhibited a slight negative correlation with time which was not significant ($p > 0.05$) (Table 2). The highest absolute growth rate (steepest regression slope) was 0.469 g·d$^{-1}$ for control fish; the corresponding gain in absolute length was 1.327 mm·d$^{-1}$ (Table 2). The mean weight gain for the four treatment groups of fish over the experimental period (Fig. 2) showed a concentration-dependent trend of decreasing total weight gain with increasing concentration of chloramine-T, although this was not statistically significant. There was no significant change in *K*-factor for any of the groups of fish over the experimental period (Table 2). This notwithstanding, the difference in appearance of the fish was striking. Fish treated with 10 and 20 mg chloramine-T·L$^{-1}$ appeared slightly emaciated despite the *K*-factor equity and often exhibited a dark colouration compared with the controls (Fig. 3A). The absolute growth rate for each treatment (slope of each regression) was compared with the control growth rate (slope) using a *t*-test. There were no significant differences in absolute growth rate between the control and those for 5 and 10 mg chloramine-T·L$^{-1}$ for weight, length, and weight/length data. However, a significant difference was observed between the absolute growth rates (regression slopes) of the control fish and those treated with 20 mg chloramine-T·L$^{-1}$ (Table 2). The slopes of the *K*-factor did not differ among any of the treatments.

## Caudal Fin Lesions

After 1 wk of chloramine-T treatments, approximately half of the fish sampled that had been exposed to 10 and 20 mg chloramine-T·L$^{-1}$ were noted to have some erosion of the caudal fin. The extent of the lesions varied from a mild state of erosion of the tissue between the fin rays (Fig. 3B) to a severe erosion of the entire fin. Progression of the lesion resulted in a necrotizing dermatitis of the caudal peduncle with zones of exposure of the underlying muscle. Fish with severely eroded caudal fins and peduncle would

TABLE 3. Bacterial isolates cultured from erosive caudal lesions. 0, no colonies present; +, low number of colonies; ++, moderate number of colonies; +++, high number of colonies.

| Bacterial isolates | Chloramine-T (mg·L$^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 5 | 10 | 20 |
| *Pseudomonas* spp. | + | ++ | +++[a,b] | +++ |
| *Flavobacter* spp. | ++ | ++ | ++ | +++ |
| *Acinetobacter* spp. | ++ | ++ | + | + |
| *Staphylococcus* spp. | ++ | 0 | + | +++ |
| *Bacillus* spp. | + | 0 | + | 0 |
| *Aeromonas hydrophila* | 0 | 0 | +++[b] | ++[b] |

[a] Including one isolation of *Pseudomonas fluorescens*.
[b] Detected only in last week of the experiment.

swim erratically, often losing equilibrium. Mortalities were observed only in the last week of the experiment as a result of this condition. As a consequence of these mortalities, further chloramine-T treatments were suspended after day 22 and the experiment terminated in consideration of the animals' welfare.

There was no significant difference ($p > 0.05$) in the weight, length, or $K$-factor of the fish exhibiting the caudal erosion compared with apparently healthy individuals from the same treatment. The incidence of the caudal erosion among all the fish sampled over the duration of the experiment was highly significant ($p < 0.05$). There was a significant increase in the proportion of fish with caudal lesions exposed to 10 and 20 mg chloramine-T·L$^{-1}$ (47.5% as compared with 3.1 and 7.5% for control and fish treated at 5 mg chloramine-T·L$^{-1}$, respectively) (Fig. 4).

Several bacterial genera were isolated from the caudal lesions (Table 3). The most consistently isolated genera were *Pseudomonas*, *Flavobacter*, and *Acinetobacter*. However, one isolation of *Pseudomonas fluorescens* and two isolations of *Aeromonas hydrophila* were made in the last week of the experiment from fish treated with 10 and 20 mg chloramine-T·L$^{-1}$, respectively (Table 3). Kidney smears yielded no bacterial growth, indicating an absence of systemic disease. Histologically the lesions did not appear to be heavily colonised by bacteria, although severe necrosis and erosion of the skin was observed accompanied by a mixed inflammatory response (Fig. 5).

### Haematocrit and Blood Chemistry

Haematocrits significantly decreased in both control and experimental fish with time. Haematocrit values for control fish and fish exposed to 5 mg chloramine-T·L$^{-1}$ fluctuated with an elevation in packed cell volume (haematocrit) immediately prior to treatment with chloramine-T. This was followed by a decline in packed cell volume following two consecutive day exposures (Fig. 6). This oscillation in packed cell volume was less pronounced at days 14 and 15 and not evident by days 21 and 22 of chloramine-T exposure (Fig. 6). Fish exposed to 10 and 20 mg chloramine-T·L$^{-1}$ showed a significantly reduced haematocrit immediately following the initial chloramine-T exposure. The haematocrit continued to decline to a minimum of approximately 30 and 33% for 10 and 20 mg·L$^{-1}$, respectively (Fig. 6). However, there was no significant difference in haematocrit values between control and experimental treatments.

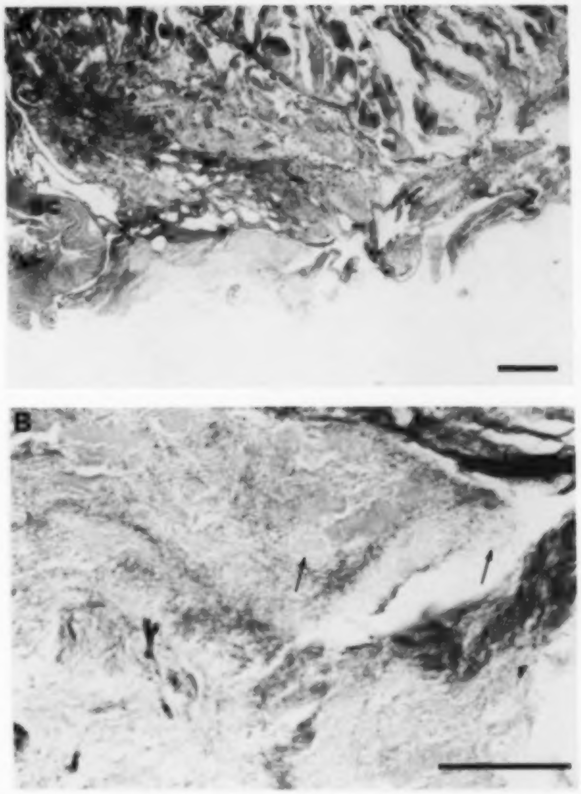

FIG. 5. (A) Erosion and necrosis around a caudal fin lesion and (B) the infiltration of numerous filamentous bacteria (arrows). sc, stratum compactum of the dermis; H & E; Bar = 200 μm.

There was a significant concentration-dependent decline in the plasma sodium ($p = 0.034$) and chloride ($p = 0.025$) concentrations of fish exposed to chloramine-T. Plasma calcium concentrations appeared to follow a similar decline (Table 4). A slight increase (although not statistically significant ($p = 0.513$)) in plasma potassium levels was also observed (Table 4). Plasma glucose levels were not significantly different ($p = 0.410$) for any of the concentrations of chloramine-T (Table 5). There was a significant ($p = 0.05$) decrease in the total plasma protein concentration in fish exposed to 10 and 20 mg chloramine-T·L$^{-1}$ (Table 5).

## Discussion

The crowding of the fish at the surface of the tank following treatment with 10 and 20 mg chloramine-T·L$^{-1}$ may have resulted in increased aggressive behaviour leading to caudal fin nipping. Lesions from this behaviour could have provided an opportunity for facultative pathogen colonisation. No single etiological agent can explain the caudal lesions, but several bacterial genera were consistently isolated. The most common isolations were of *Pseudomonas* spp. and *Flavobacter* spp. which appeared to be more prevalent in fish treated with 10 and 20 mg chloramine-T·L$^{-1}$. These genera are ubiquitous in freshwater and have been considered to be part of the normal surface bacterial flora of rainbow trout skin (Horsley 1977). These increased numbers of bacteria are correlated with the increased incidence of the lesions

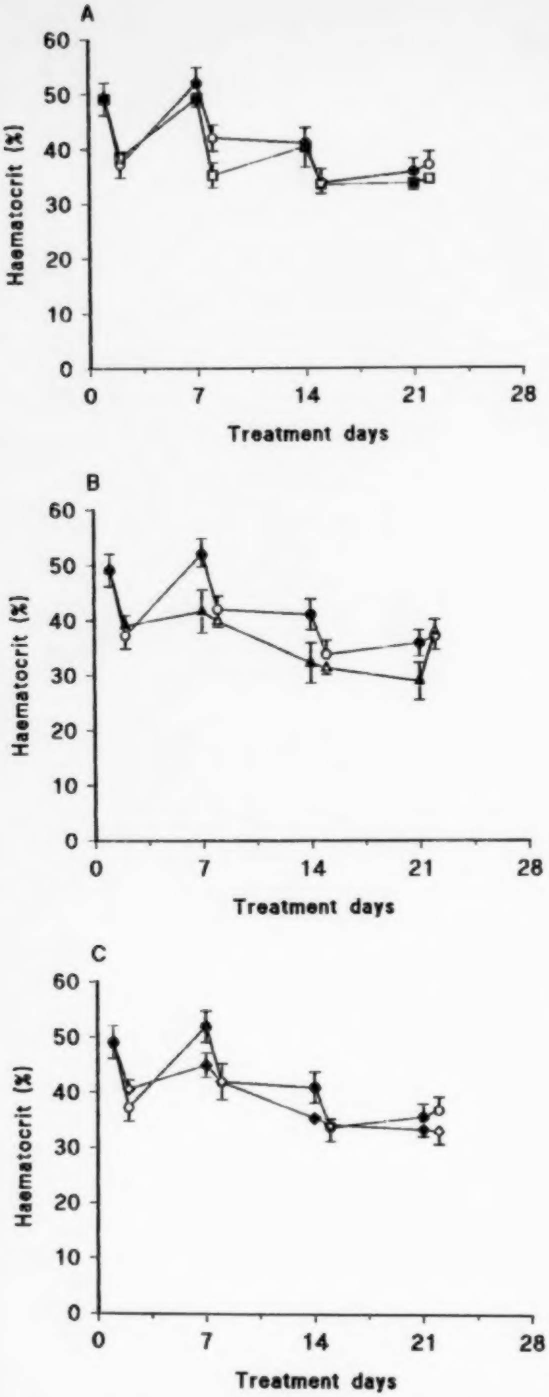

FIG. 6. Mean haematocrit (+SEM) profiles for trout intermittently treated with chloramine-T. Solid symbols represent mean haematocrit prior to weekly exposure and open symbols represent mean haematocrit following weekly exposures; circles represent control values. (A) 5 mg chloramine-T$\cdot$L$^{-1}$ (squares); (B) 10 mg chloramine-T$\cdot$L$^{-1}$ (triangles); (C) 20 mg chloramine-T$\cdot$L$^{-1}$ (diamonds).

in fish treated with higher dosages of chloramine-T in this investigation. The opportunistic pathogen load may have been a significant factor in initiating or promoting the progression of the dermal lesions, but although the isolations (Table 3) show an apparent association, they do not prove or diagnose a causal role.

A decrease in plasma sodium and chloride levels is indicative of osmoregulatory compromise (Wedermeyer et al. 1991). This would result in haemodilution, hence the reduction in total plasma protein concentration. This haemodilution may have been caused by the dermatitis. However, it cannot be discounted that chloramine-T may have also had an effect on the gill either by causing epithelial degeneration like that described for chlorine on trout gills as reported by Bass et al. (1977) or by impairment of the function of the ion regulation at the membranes of pavement epithelial and chloride cells (Goss et al. 1992a, 1992b). Possible damage to chloride cells is also indicated by a decrease in plasma chloride and calcium concentrations (Wedemeyer et al. 1991; Perry et al. 1992). In a companion study of the gills of the fish used in this study (M.D. Powell, Wight, and D.J. Speare, unpublished data), we have demonstrated that morphological changes in the branchial epithelium are consistent with compensatory changes to facilitate increased ion exchange. Changes in haematocrit and blood chemistry in the present study were not consistent with previous studies on chlorine toxicity. Zeitoun (1977, 1978a, 1978b) described an increase in haematocrit and in plasma protein concentration as a consequence of chlorine exposure. The responses described by Zeitoun (1977, 1978a) cannot be discounted as being severe stress responses, especially when similar results were observed with mortalities from rainbow trout exposed to heated chlorinated wastewater from a power generating plant (Zeitoun 1978b). Fish under stress have shown an elevation in haematocrit (Casillas and Smith 1977) as well as elevations in plasma cortisol and glucose concentrations (Pickering 1992). Zeitoun (1977, 1978a, 1978b) did not measure plasma cortisol or glucose concentrations. In our study, there was no elevation in plasma glucose concentration following chloramine-T exposure at any of the experimental dosages used in this study, nor was there any increase in haematocrit following treatment. This would indicate an absence of an acute adrenergic stress response (Mazeaud et al. 1977) due to either the exposure of the fish to chloramine-T or handling stress during the sampling procedure. Secondary responses resulting from prolonged stress and elevated plasma cortisol levels ultimately lead to gluconeogenesis and subsequent elevated plasma glucose levels (Barton et al. 1986, 1987). This secondary stress response of elevated plasma glucose concentrations was not evident in our study. The fish exposed to chloramine-T (particularly those exposed to 10 and 20 mg$\cdot$L$^{-1}$) did exhibit behavioural changes (crowding at the water surface, hyperventilating during chloramine-T exposure, darkening in colouration) which were thought to be responses to acute hypoxic stress. The nature of this hypoxia may have been due to an irritation of the branchial membranes leading to a hypersecretion of mucus, as was suggested by Bass et al. (1977) who observed similar ventilatory changes upon exposure of trout to chlorine (Bass and Heath 1977). However, these signs subsided within 24 h, suggesting that the irritant effects of chloramine-T subsided rapidly and were insufficient to ellicit a compensatory increase in haematocrit. Owing to the small amount

TABLE 4. Plasma ion concentrations (mean ± SEM) for rainbow trout following 4 wk intermittent exposure to chloramine-T.

| Chloramine-T concentration ($\mathrm{mg \cdot L^{-1}}$) | Plasma concentration ($\mathrm{mmol \cdot L^{-1}}$) | | | |
|---|---|---|---|---|
| | Na$^+$ | K$^+$ | Cl$^-$ | Ca$^{2+}$ |
| 0 | 148.50 ± 1.06 | 5.28 ± 0.45 | 115.25 ± 1.73 | 2.10 ± 0.12 |
| 5 | 146.00 ± 2.85 | 5.45 ± 0.84 | 113.75 ± 1.77 | 2.28 ± 0.06 |
| 10 | 137.25 ± 3.80[a] | 6.45 ± 1.15 | 106.75 ± 2.97[a] | 1.79 ± 0.30 |
| 20 | 137.00 ± 2.10[ab] | 6.93 ± 0.50 | 105.50 ± 1.48[ab] | 1.67 ± 0.11[ab] |

[a] Significantly different from control at $p < 0.05$.
[b] Significantly different from 5 $\mathrm{mg \cdot L^{-1}}$ at $p < 0.05$.

of blood that could be collected from the fish in our study, it was not possible to examine plasma cortisol concentrations.

The apparent suppression of growth rate with chloramine-T treatment (Table 2) must be interpreted with caution. There are many factors that may influence growth in fish (Lanno et al. 1989). This study was not designed to dissect the mechanism of growth suppression; rather it was to determine if there was a negative effect of repeated chloramine-T treatment on growth. The suppression of growth was only significant at the highest dose of chloramine-T. This may be due to reduced feed intake as a consequence of stress or to reduced feed conversion. Our observations indicated that there was no apparent difference in the amount of uneaten feed from any of the tanks. Unfortunately this was not quantified. Perhaps if the study could have been continued beyond 4 wk and the sample size increased, significant growth suppression and change in the condition of the fish might have been detected. There was no apparent difference in the condition (*K*-factor) of any of the groups of fish. This may have been due to the time frame of this investigation. According to Busacker et al. (1990), the *K*-factor is most appropriately used for measuring growth over a period of weeks to months. However, extension of this study was limited due to the occurrence of the bacterial caudal erosive dermatitis. Continued treatment may have ultimately led to a reduction in condition of treated fish, as the difference in weight gain and length between control and treated fish increased. There was some evidence of this beginning to occur, since the fish treated at the 10 and 20 mg chloramine-T$\cdot$L$^{-1}$ dosages appeared emaciated due to either difference in conversion efficiency or reduced feed intake. Alternatively, fish treated with chloramine-T may have exhibited substantial compensatory growth if the experiment had been continued. More longer term studies are obviously required to elucidate the answers to some of these questions. Although the differences in mean weight change (Fig. 2) were not statistically significant, a concentration-dependent trend was apparent. Significance may have been masked by the variation in size of the fish used and small sample size ($n = 4$ controls and $n = 5$ treatments). Nevertheless, an apparent reduction in growth rate even in the short term is of significant importance to the aquaculture industry.

In conclusion, we have demonstrated that an intermittent prophylactic-like treatment regime of chloramine-T exposure at concentrations used in the aquaculture industry may induce an ionoregulatory compromise and perhaps a stress-related suppression of growth. Chloramine-T exposure appeared to increase the susceptibility to an erosive dermatitis of the caudal fin and peduncle. This disease may have been mediated or accentuated by opportunistic pathogens of the genus *Pseudomonas* or *Flavobacter* bacteria following changes in behaviour of the fish or as a consequence of chloramine-T associated treatment stress. However, blood chemistry changes following four consecutive weeks of chloramine-T exposure were not consistent with either a chronic stress response or previous studies on chlorine toxicity. Reductions in plasma sodium, chloride, and calcium concentrations suggest an ionic and/or osmotic compromise. Suppression of haematocrit and total plasma protein concentration would suggest hemodilution. These changes in blood chemistry and haematocrit may have arisen as a consequence of chloramine-T-induced ionoregulatory disturbance causing changes in the gill function, especially that of chloride cells. Alternatively, chloramine-T may have been caused by damage to the epithelium of the skin that ultimately manifested in the erosive lesions of the caudal fin and peduncle. Further work is required to dissect the mechanism by which growth was suppressed and apparent ion regulatory compromise mediated. Nevertheless, this study demonstrates that chloramine-T should perhaps be used with caution, and if used for prophylactic-like repeated exposures, then concentrations should be lower than those recommended for therapeutic use of the drug. Such doses should be around 5 $\mathrm{mg \cdot L^{-1}}$ to minimize possible effects on growth and ion regulation.

TABLE 5. Total blood protein and plasma glucose concentrations for rainbow trout following 4 wk of intermittent exposure to chloramine-T.

| Chloramine-T concentration ($\mathrm{mg \cdot L^{-1}}$) | Total plasma protein ($\mathrm{g \cdot L^{-1}}$) | Plasma glucose ($\mathrm{mmol \cdot L^{-1}}$) |
|---|---|---|
| 0 | 29.00 ± 1.71 | 3.23 ± 0.18 |
| 5 | 27.00 ± 0.93 | 3.77 ± 0.23 |
| 10 | 18.00 ± 3.85[a] | 3.03 ± 0.48 |
| 20 | 20.00 ± 1.65[ab] | 3.73 ± 0.14 |

[a] Significantly different from control at $p < 0.05$.
[b] Significantly different from 5 $\mathrm{mg \cdot L^{-1}}$ at $p < 0.05$.

## Acknowledgements

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# Modelling Pelagic Fish and Plankton Trophodynamics off Southwestern Vancouver Island, British Columbia

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Robinson, C.L.K., and D.M. Ware. 1994. Modelling pelagic fish and plankton trophodynamics off southwestern Vancouver Island, British Columbia. Can. J. Fish. Aquat. Sci. 51: 1737–1751.

A trophodynamics model is used to estimate annual plankton and fish production for the southern British Columbia continental shelf during 1985–89. The model describes the feeding interactions among diatoms, copepods, euphausiids, juvenile and adult Pacific herring (*Clupea pallasi*), Pacific hake (*Merluccius productus*), chinook salmon (*Oncorhynchus tshawytscha*), and spiny dogfish (*Squalus acanthias*) and is forced by empirical seasonal patterns in upwelling, sea surface temperature, and solar radiation. The most important simulation results are that (1) there is an imbalance between fish consumption and euphausiid production during the summer upwelling season, (2) the biomass and arrival timing of migratory hake significantly influence plankton and fish production, and (3) about 11% of the 332 g $C{\cdot}m^{-2}{\cdot}yr^{-1}$ annual diatom production is transferred to copepods and euphausiids and 1.0% of the diatom production to fish, while 27.5% of the 11.9 g $C{\cdot}m^{-2}{\cdot}yr^{-1}$ euphausiid production is consumed by herring and hake. The high plankton and fish production on the southern British Columbia shelf is comparable with other productive coastal upwelling regions.

On fait appel à un modèle trophodynamique pour évaluer la production annuelle de plancton et de poisson sur la plate-forme continentale du sud de la Colombie-Britannique entre 1985 et 1989. Le modèle décrit les interactions alimentaires entre les diatomées, les copépodes, les euphausiacés, les harengs du Pacifique (*Clupea pallasi*) juvéniles et adultes, le merlu du Pacifique (*Merluccius productus*), le saumon quinnat (*Oncorhynchus tshawytscha*) et l'aiguillat commun (*Squalus acanthias*) et on le force à utiliser les données saisonnières empiriques de remontée des eaux, les données de température à la surface de la mer et les données de rayonnement solaire. Les plus importants résultats de la simulation sont les suivants : 1) il y a déséquilibre entre la consommation de poissons et la production des euphausiacés au cours de la saison de remontée des eaux en été, (2) la biomasse et le moment de l'arrivée des merlus en migration ont une influence significative sur la production de plancton et de poisson et (3) environ 11% de la production annuelle de diatomées (332 g $C{\cdot}m^{-2}{\cdot}an^{-1}$) est transférée aux copépodes et aux euphausiacés et 1,0% de la production de diatomées est transférée aux poissons, alors que 27,5% de la production d'euphausiacés (11,9 g $C{\cdot}m^{-2}{\cdot}an^{-1}$) est consommée par le hareng et le merlu. La production élevée de plancton et de poisson sur la plate-forme continentale du sud de la Colombie-Britannique se compare à celle d'autres régions côtières de remontée des eaux productives.

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The continental shelf off southern Vancouver Island is the most productive fishing region along the west coast of Canada. During the last decade, the annual catch of commercial fish around La Perouse Bank has averaged 5–6 tonnes $(t){\cdot}km^{-2}$ (Fig 1; Ware and McFarlane 1989). The high annual fishery yields from this region are related to seasonal aggregations of fish and to high plankton production. The latter results from upwelling of nutrients and transport of nutrients and plankton from Juan de Fuca Strait and from coastal northern Vancouver Island (Mackas et al. 1987; Ware and Thomson 1991).

To better understand the fish production potential off southern Vancouver Island, the major pathways of biomass transfer among abundant pelagic organisms need to be rigorously evaluated. In studies of marine systems, ecological transfer efficiencies have been used to estimate fish production from primary production (e.g., Ryther 1969). This approach is limited because of potential temporal variability and large uncertainties in transfer efficiencies and primary production and because the magnitudes of additional losses among trophic levels are relatively unknown (Parsons et al. 1984; Iverson 1990). An alternative approach estimates fish production by relating what and how many prey are eaten by dominant fishes. In turn, the resources needed to sustain prey biomasses are evaluated (e.g., Laevastu et al. 1982). This trophodynamical approach has the advantage of estimating lower bounds of production and standing stocks from data describing the feeding requirements of dominant organ-

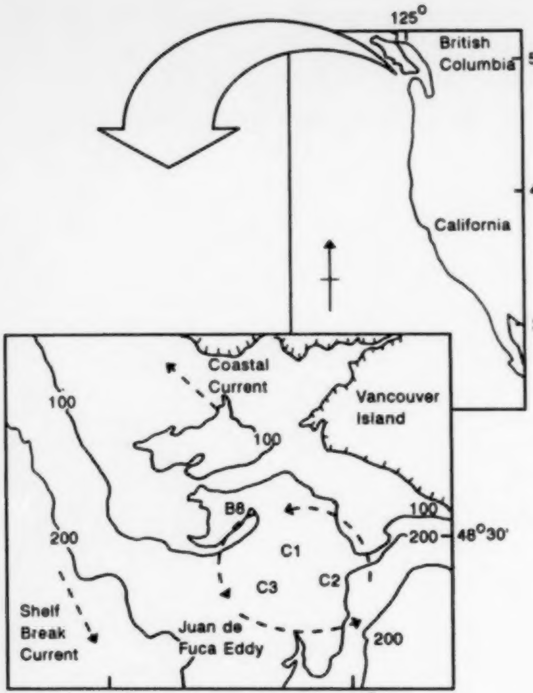

FIG. 1. Location of zooplankton sampling stations (C1, C2, C3, and B8) in the Juan de Fuca Eddy region off southwestern Vancouver Island. Broken arrows indicate major surface currents during the summer. Depths in metres (after Thomson and Ware 1988; Mackas 1992).

isms. Prey biomasses and production rates can also be related to changes in other prey and predators (i.e., allows for multispecies effects) and to nontrophodynamical losses.

One of the major trophodynamical links between plankton and pelagic fish in coastal upwelling regions occurs via net phytoplankton-to-meso/macrozooplankton-to-fish (Ryther 1969; Cushing 1989). The predominance of this pathway is related to the fact that there is a relatively constant predator-to-prey size ratio in aquatic food chains. For example, phytoplankton <5–10 μm are primarily eaten by protozoans, while larger phytoplankton (e.g., diatoms) are primarily eaten by metazoan zooplankters, like copepods, because the latter are inefficient consumers of small particles (Parsons et al. 1984; Legendre and LeFevre 1992). The diatom–metazoan pathway ultimately predominates in productive coastal systems because diatoms generally dominate phytoplankton biomass (Kiorboe 1993).

The relative importance of the diatom – meso/macro zooplankton – fish pathway on the southern British Columbia (B.C.) continental shelf is supported by observations that during the upwelling season, phytoplankton are dominated by diatom genera such as *Rhizosolenia* or *Nitzschia* (Mackas and Sefton 1982). About 60–80% of the meso/macro zooplankton biomass consists of large neritic calanoid copepods (e.g., *Calanus marshallae*) and the euphausiids (*Thysanoessa spinifera* and *Euphausia pacifica*; Mackas 1992). The euphausiids, in turn, constitute the majority (60–80%) by weight of the diets of three fishes that make up >80% of the upwelling season pelagic fish biomass: Pacific hake (*Merluccius productus*), Pacific herring (*Clupea pallasi*), and spiny dogfish (*Squalus acanthias*). Chinook salmon (*Oncorhynchus tshawytscha*) are also important in this region because they consume large biomasses of small fishes such as herring (Tanasichuk et al. 1991; Ware and McFarlane 1994).

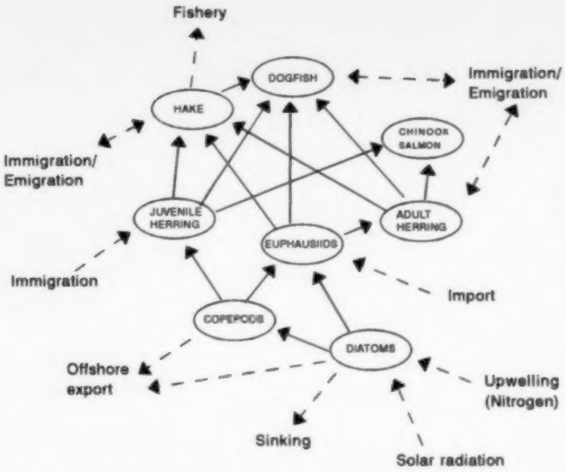

FIG. 2. Overview of the feeding interactions (solid lines and arrows) and other processes included in the Juan de Fuca trophodynamics model. Water temperature effects diatom growth, all feeding interactions, and hake biomass. Broken lines indicate nontrophodynamical effects on organism pools.

To estimate the annual production of the dominant pelagic plankton and fish on the southern B.C. continental shelf, we developed a simulation model describing the trophodynamics of this upwelling system. The simulation model results from combining two existing models. The first model (Robinson et al. 1993) describes feeding interactions among diatoms, copepods, and euphausiids on the B.C. continental shelf as influenced by seasonal cycles in upwelling, water temperature, and solar radiation. This model is limited in scope because it uses a generalized, time-dependent fish feeding function. The second model (developed by D. Ware, unpublished) examines feeding interactions among dominant pelagic fishes on the B.C. continental shelf. This model is limited because it simplifies euphausiid production and only considers feeding interactions during the upwelling season.

Several improvements in modelling the trophodynamics of plankton and fish on the continental shelf off southwestern B.C. are made here by coupling the Robinson et al. (1993) plankton model and Ware's fish model. These improvements include year-round feeding interactions, temperature ($Q_{10}$) effects on all feeding interactions, and increased complexity of feeding interactions among organisms. The main objectives of this paper are to compare standard model output with empirical biological data for 1985–89 and to describe general system production properties.

## Model Overview

During the productive April–October upwelling season, the southern B.C. continental shelf consists of at least three major oceanographic and biological subregions (Mackas 1992). We are interested in the oceanic subregion characterized by an upwelling-induced quasi-permanent cyclonic eddy, the Juan de Fuca Eddy (Fig. 1; Freeland and Denman 1982). Compared with surrounding oceanographic subregions, the Juan de Fuca Eddy typically contains the highest concentrations of zooplankton, particularly large calanoid copepods and euphausiids; gelatinous zooplankton are less important in this subregion (Mackas 1992). The Eddy region also contains the largest concentrations of migratory fishes like Pacific hake, as well as resident species such as Pacific herring, spiny dogfish, and chinook salmon (Tanasichuk et al. 1991; Ware and McFarlane 1994). The main concentrations of fish occur in and around the deep basins (100–200 m) of the Eddy region, an area of roughly 1520 $km^2$.

Since the Eddy is a relatively large and complex oceanic region, we ignored detailed horizontal and vertical spatial resolution in the model. The only generalized effect of horizontal water movement considered is the export of diatoms and copepods from the Eddy to offshore regions (Mackas 1992). In addition, euphausiids are assumed to be imported into, and accumulated in, the Eddy region. Both import and export of plankton are related to the pattern and intensity of Ekman transport (Robinson et al. 1993).

## Model Processes

The major processes included in the Juan de Fuca Eddy trophodynamics model are described in Fig. 2 and discussed below and in Appendix 1.

### *Plankton dynamics*

Diatom growth in the model is influenced by water temperature and limited by the combined effects of nitrogen and light using a Michaelis–Menten equation. Diatoms are grazed by both copepods and euphausiids and are exported to offshore waters as a function of the intensity and pattern of Ekman transport. The sinking of diatoms from the surface mixed layer is inversely related to nitrogen concentration (Appendix 1).

Copepods feed exclusively on diatoms and are consumed by euphausiids and juvenile herring. Copepods are exported from surface waters of the Eddy region to offshore waters by Ekman transport. Euphausiids consume both copepods and diatoms and are subsequently eaten by hake, herring, and dogfish (Tanasichuk et al. 1991; Ware and McFarlane 1994). Euphausiids are not exported from the Eddy region because they vertically migrate below the surface offshore Ekman layer (Simard and Mackas 1989). Euphausiids are imported into, and accumulate in, the Eddy region (Thomson and Ware 1988; Robinson et al. 1993).

### *Fish dynamics*

Hake biomass, in thousands of tonnes (kt), off southern Vancouver Island is estimated from a temperature-dependent function derived by Ware and McFarlane (1994): hake biomass (kt) = average June and July SST·196.428 − 2231.771 where SST is the sea surface temperature at Amphitrite Point, B.C. We assume that 90% of the estimated hake biomass immigrates into the Eddy region at a constant rate over 30 d starting June 1. Hake emigration from the Eddy to offshore regions during summer/fall is assumed to be inversely related to euphausiid biomass in the Eddy region. Hake feed on euphausiids and juvenile and adult herring and in turn are eaten by dogfish (Ware and McFarlane 1994). Hake are removed by a fishery at a constant rate (427 $t \cdot d^{-1}$) from June 1 to October 31.

Pacific herring are divided into adults (>3+ yr) and juveniles (0, 1+, and 2+ yr). Ware and McFarlane (1994) estimated that 55 kt of adult herring were found off southern Vancouver Island during the 1980s. Adult herring immigrate to the Eddy region from nearshore spawning areas (e.g., Barkley Sound and the Strait of Georgia) at a constant rate over 30 d starting April 1. Adult herring also emigrate at a constant rate out of the Eddy region in late December to form prespawning aggregations in the sounds along the lower west coast of Vancouver Island and the Strait of Georgia. Adult herring feed exclusively on euphausiids and are eaten by hake, dogfish, and chinook salmon (Appendix 1).

Juvenile herring are included in the model because they represent a large biomass of small fishes that feed on copepods. Based on preliminary calculations, we estimate that the juvenile herring biomass is roughly equivalent to the adult herring biomass (55 kt). One half of the 55-kt juvenile biomass occurs in the Eddy region year-round, while the remaining biomass of juvenile herring (young-of-the-year) immigrates into the Eddy region from nearshore nursery areas in mid-August (Ware and McFarlane 1994). In the model, juvenile herring feed on copepods and are eaten by hake, dogfish, and chinook salmon.

Dogfish biomass in the Eddy region is roughly 50% of the 38 kt estimate made for the west coast of Vancouver Island (Ware and McFarlane 1994). Ketchen (1986) noted a seasonal movement of dogfish along the coasts of B.C. and Washington. We model the movement of dogfish into and out of the Eddy region by linking it to the seasonal cycle in water temperature. Maximum dogfish biomass (20 kt) occurs in August, while minimum concentrations (10 kt) occur in January. Dogfish consume hake, both groups of herring, and euphausiids (Tanasichuk et al. 1991).

Eleven thousand tonnes of Chinook salmon are assumed to feed exclusively on adult and juvenile herring year-round (M. Healy, University of British Columbia, Vancouver, B.C., unpublished data).

### *Feeding functions*

In the model, the ingestion of plankton by plankton and zooplankton by fish is simulated using a generic saturating function $I \times Q_{10} \times ((P - P_t)/(P - P_t + H_p))$ where $I$ is a predator-dependent maximum ingestion rate, $Q_{10}$ results in a doubling of the ingestion rate for every 10°C increase in water temperature, $P$ is the prey concentration, $P_t$ is a prey threshold concentration below which predators cease feeding, and $H_p$ is the prey concentration at which predator ingestion is one half the maximal rate. Prey threshold concentrations are estimated as observed minimum prey concentrations (e.g., winter diatom, copepod, and euphausiid concentrations). Half-saturation concentrations are 1.5–2 times greater than threshold values (See Appendix 1 and Table 1 for specific ingestion parameter values).

The ingestion of euphausiids by hake does not include a prey threshold concentration or a half-saturation constant. Rather, empirical data suggested that the hake ingestion rate is positively related to euphausiid concentration (as an exponential-saturating function) and inversely to the hake emigration rate. The hake ingestion function is similar to a hyperbolic ingestion function in that both result in reduced

TABLE 1. List of state variables and parameter values used to generate Fig. 3, 4, and 5. Subscripts refer to state variables. Other abbreviations: DW, dry weight; %BWD, percent body weight per day. References: 1, Robinson et al. (1993); 2, Ware and McFarlane 1994; 3, this study.

| Variable | Description | Units | Value | Reference |
|---|---|---|---|---|
| | *Starting parameters* | | | |
| $N$ | Nitrogen | $\mathrm{mmol \cdot m^{-3}}$ | 4.0 | 1 |
| $D$ | Diatoms | mg Chl $a \cdot \mathrm{m^{-3}}$ | 0.5 | 1 |
| $C$ | Copepods | $\mathrm{g\ DW \cdot m^{-2}}$ | 0.75 | 1 |
| $E$ | Euphausiids | $\mathrm{g\ DW \cdot m^{-2}}$ | 2.0 | 1 |
| $J$ | Juvenile herring (Jan.) | kt | 28 | 2 |
| | Juvenile herring (Aug.) | kt | 28 | 2 |
| $H$ | Adult herring | kt | 55 | 2 |
| $W$ | Hake | kt | 210 | 2 |
| $S$ | Dogfish | kt | 20 | 2 |
| $O$ | Salmon | kt | 11 | 2 |
| $A_W$ | Arrival of hake | d | 152 | 1, 3 |
| $A_J$ | Arrival of juvenile herring | d | 227 | 3 |
| $A_H$ | Arrival of adult herring | d | 91 | 3 |
| | *Plankton parameters* | | | |
| $T_d$ | Diatom export rate | $\mathrm{d^{-1}}$ | 0.55 | 1 |
| $S_d$ | Diatom sinking rate | $\mathrm{m^{-1}}$ | 1.0 | 1 |
| $R_d$ | Diatom respiration rate | $\mathrm{d^{-1}}$ | 0.05 | 1 |
| $H_n$ | Nitrogen half-saturation constant | $\mathrm{mmol \cdot m^{-3}}$ | 1.0 | 1 |
| $T_c$ | Copepod export rate | $\mathrm{d^{-1}}$ | 0.13 | 1 |
| $A_c$ | Copepod availability | % | 40.0 | 1 |
| $I_c$ | Copepod max. ingestion | %BWD | 50.0 | 1 |
| $G_c$ | Copepod growth efficiency | % | 40.0 | 1 |
| $I_e$ | Euphausiid max. ingestion | %BWD | 20.0 | 1 |
| $G_e$ | Euphausiid growth efficiency | % | 25.0 | 3 |
| $R_e$ | Euphausiid import | $\mathrm{g\ DW \cdot m^{-2} \cdot d^{-1}}$ | 0.07 | 3 |
| | *Fish ingestion parameters* | | | |
| $I_j, I_h, I_s, I_w$ | Max. ingestion rates | %BWD | 3, 1.6, 2, 4 | 3 |
| $G_j, G_h, G_w$ | Growth efficiencies | % | 20, 20, 10 | 3 |
| $H_j, H_h, H_s$ | Prey half-saturation | kt | 50, 30, 40 | 3 |
| $P_j, P_h, P_s$ | Prey thresholds | kt | 17, 20, 20 | 3 |

predation rates when prey concentrations are low and increased predation at higher prey concentrations.

The ingestion of adult and juvenile herring by fish is inversely related to euphausiid consumption by the predator (Ware and McFarlane 1994). At most, 60 and 40% of the hake and dogfish herring ration consists of adult and juveniles, respectively. The chinook salmon herring ration comprises 80% adults and 20% juveniles (Shaw et al. 1989).

Ingested prey are converted to predator biomass using estimates of predator-specific gross growth efficiencies. Gross growth efficiency (GGE) is defined as the proportion of ingested prey that is instantaneously converted into somatic and reproductive tissue (Parsons et al. 1984). For copepods and euphausiids, GGE varies between maximum positive and minimum negative values according to prey concentrations (Robinson et al. 1993). Because empirical data describing fish saturating and maintenance rations are not available, we assume that GGEs are constant over time. The maximum GGE for hake and herring is estimated from observed maximum growth ($G$) and estimated ingestion ($I$) rates ($G/I$ = GGE). Biomass growth is not explicitly modelled for salmon or dogfish because of small biomass and slow growth rates, respectively.

*Environmental forcing*

The model is forced with seasonal patterns in three oceanic functions: Ekman transport, sea surface temperature, and solar radiation, observed from 1985 to 1989. Ekman transport influences diatom growth via nutrient input to the surface layer, exports plankton biomass offshore, and imports euphausiids. Water temperature influences diatom growth rate and all ingestion rates, while solar radiation influences the growth of diatoms (Fig. 2; Appendix 1).

## Results and Discussion

### Zooplankton Biomass Patterns

The simulated copepod and euphausiid biomass patterns are calibrated to empirical patterns derived for the Eddy region during 1985–89. We derived empirical seasonal patterns for zooplankton from data collected by bongo net (0.23-mm mesh) at three stations over deepwater basins of the Eddy (C1, C2, C3) and an additional basin station (B8) located northeast of La Perouse Bank (Fig. 1; Mackas 1992). Combining B8 with the three Eddy stations is justified because all four basin stations have similar zooplankton species

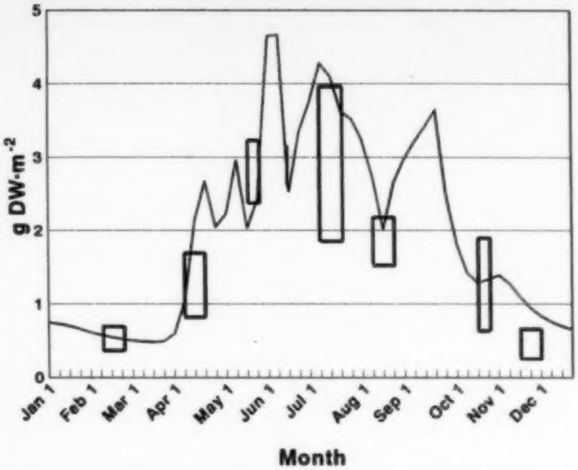

FIG. 3. Simulated copepod biomass calibrated to 1985–89 empirical data. Boxes indicate ±1 SD in sampling time and absolute concentrations.

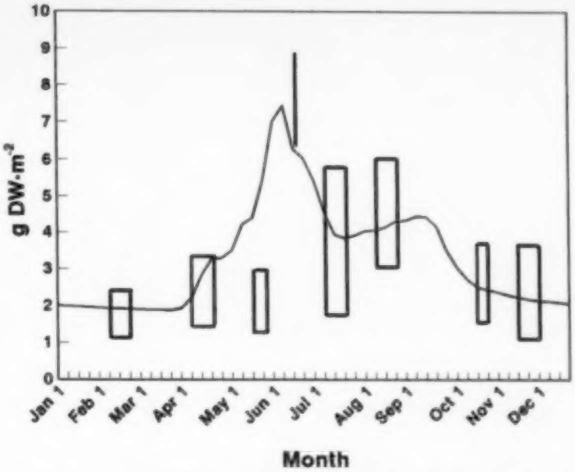

FIG. 4. Simulated euphausiid biomass calibrated to 1985–89 empirical data. Boxes indicate ±1 SD in sampling time and absolute concentrations.

composition (see fig. 4 in Mackas 1992). The available samples collected at the four stations are pooled for each month, and arithmetic averages are calculated. Data for *C. marshallae* and *Pseudocalanus* spp. are combined to describe seasonal changes in copepod biomass, while data for *T. spinifera* are used to describe changes in euphausiid biomass.

*Copepods*

The copepod simulation captures the observed spring and early summer increase in copepod biomass as a direct result of growth from increased diatom production (Fig. 3). The general decline in copepod biomass during the summer is produced by the effects of offshore transport, as hypothesized by Mackas (1992). Initially, however, simulated copepod biomass in late summer remains substantially higher than observed data even with offshore transport. In nature, this period corresponds to the arrival of a large biomass of juvenile herring to the La Perouse Bank region (Ware and McFarlane 1994). Inclusion of juvenile herring feeding on copepods more closely calibrates model copepods to the empirical data (Fig. 3).

*Euphausiids*

Observed biomasses of *T. spinifera* measured at the Eddy stations during 1985–89 are relatively low from May to July, before increasing to maximum concentrations from late August to October (see also Mackas 1992). However, in Appendix 2, we present several independent lines of evidence that indicate that the empirical pattern may not be fully capturing the seasonal dynamics in euphausiid biomass, particularly in late spring or early summer. We use the "adjusted" euphausiid biomass pattern discussed in Appendix 2 to calibrate model euphausiids rather than the pattern discussed by Mackas (1992) because we believe the former is more consistent with available evidence.

Initially, the simulated euphausiid biomass pattern did not calibrate very well to the adjusted empirical seasonal biomass pattern, nor did it meet the late winter/early spring or summer grazing demands of the fish. Robinson et al. (1993) and Robinson (1994) determined that a relatively high average adult euphausiid growth rate (>3% body weight·d$^{-1}$; BWD) and enhancement of euphausiid biomass by oceanographic mechanisms are both required to calibrate the simulated biomass pattern to empirical data. The idea that physical oceanographic mechanisms accumulate zooplankton biomass in coastal regions is not new. For instance, several studies in the coastal Benguela upwelling system have indicated intense interactions between coastal current dynamics and euphausiids (Barange and Pillar 1992). It is also apparent that when oceanographic mechanisms break down, euphausiid aggregations "dissolve", although this process can be complicated by food-web interactions (Harris et al. 1992).

On the southern B.C. shelf, adult and juvenile euphausiids could be "imported" from upwelling regions along the continental shelf–slope interface, from deepwater input via Spur canyon, and/or juvenile stages from Juan de Fuca Strait outflow (Ware and Thomson 1986; Simard and Mackas 1989; Mackas 1992; Ware and McFarlane 1994). Consequently, older stages of euphausiids would be retained in the Eddy region because of their diel vertical migratory behaviours and because of the retentive circulation mechanisms associated with the cyclonic gyre (Freeland and Denman 1982).

To explore the possible importance of euphausiid import, we linked this process to the seasonal pattern and intensity of upwelling as inferred from positive Ekman transport. Maximum import occurs during the week of greatest Ekman transport and is made proportional to the remaining weeks during the upwelling season. Simulations demonstrate that the euphausiid import process results in a temporal biomass pattern that calibrates very well to the adjusted empirical data (Fig. 4). Simulated maximum euphausiid growth rates and seasonal hake ingestion patterns are also consistent with empirical data (see Fig. 6).

Combined, import and high average growth of euphausiids allow their biomass to increase fourfold from late April to early June. By mid-July however, there is a relatively rapid decline in empirical and simulated euphausiid biomass to roughly one half that of the mid-June peak (Fig. 4). This rapid decline is due primarily to predation by hake. The

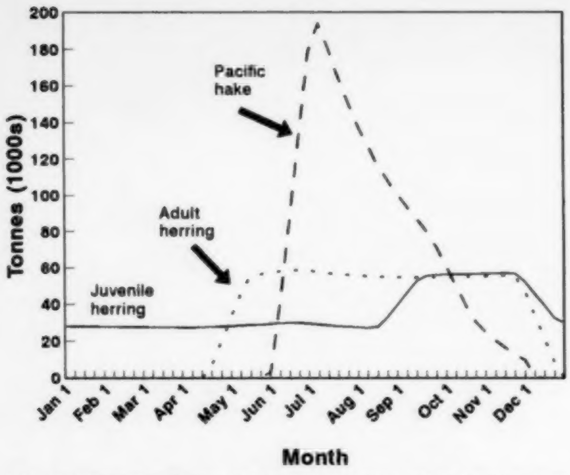

FIG. 5. Biomass patterns of juvenile herring, adult herring, and Pacific hake as determined for the 1985–89 simulation.

empirical and simulated data further indicate an increase in euphausiid biomass from late July to early September (Fig. 4). This increase can also be linked to the effects of fish predation. As euphausiid biomass declines in the Eddy region, hake begin to emigrate to the shelf break in search of more abundant supplies of prey (Ware and McFarlane 1994). Thus, fish predation is reduced and growth and import continue to increase euphausiid biomass.

The simulated and observed data also indicate that there is roughly a twofold decline in euphausiid biomass from early September until mid-November (Fig. 4). This decline is due to the cessation of euphausiid import by the end of September and to slight negative growth associated with declining prey production. Both processes result from relaxation in upwelling and the concomitant increase in downwelling.

### Fish Biomass Patterns

Hake biomass reaches a maximum of about 200 kt by the end of June and declines during July and August (Fig. 5). The late-summer decline in hake biomass is due to emigration from the Eddy region (linked to euphausiid biomass) and fishing. The decline in Eddy region hake biomass is consistent with observations of the hake fishing fleet moving further offshore in pursuit of hake (Ware and McFarlane 1994). About 100 kt of hake is present in late August, but biomass continues to decline until late October when no hake remain in the Eddy region (Fig. 5).

To further ensure the efficacy of the hake biomass pattern, we calibrated model output to empirical data describing the hake ingestion rate and the fraction of euphausiids in the hake diet (Fig. 6) while at the same time ensuring that the empirical euphausiid biomass pattern was maintained (Fig. 4). Calibrating the model to these three variables generates the most realistic biomass patterns; simulations using only one variable result in different patterns of hake and euphausiid biomass.

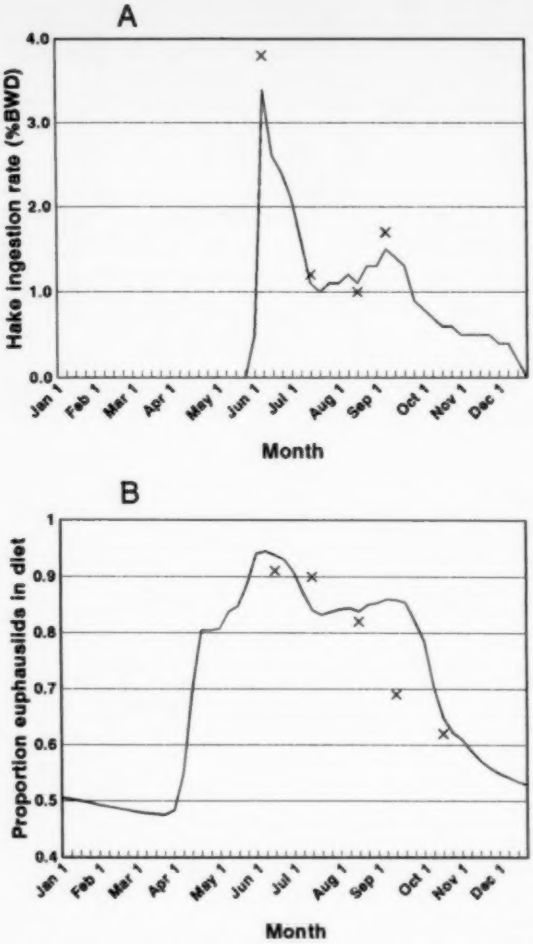

FIG. 6. (A) Simulated hake ration and (B) fraction of euphausiids in the hake diet calibrated to empirical data from Tanasichuk et al. (1991) (Crosses; both measured as volume).

Adult herring biomass increases in the Eddy region during April as they immigrate from inshore spawning grounds (Fig. 5). In May and June, adult herring biomass continues to grow as a result of feeding on euphausiids. However, during July and August, adult herring biomass declines to about 54 kt, primarily as a result of hake predation. Over the summer and fall, an average adult herring growth rate of about 0.27% BWD balances mortality from piscivores. The average model adult herring growth rate compares well with that observed by R.W. Tanasichuk and D.M. Ware (unpublished data; 0.29% BWD). Model juvenile herring need to ingest about 3% BWD of copepods to balance mortality from hake, dogfish, and salmon and to generate a copepod biomass pattern consistent with empirical data (Fig. 3).

### Sensitivity Analyses

The good agreement between the standard-run and the empirical data is a measure of the validity of the simplifying assumptions used. Robinson (1994) has fully evaluated model sensitivity to perturbations in starting parameter values, plankton concentrations, and to environmental forcing functions. The most important result from these sensitivity analyses is that perturbations in environmental factors (e.g., upwelling rate) have a much larger impact (5–10 times) on

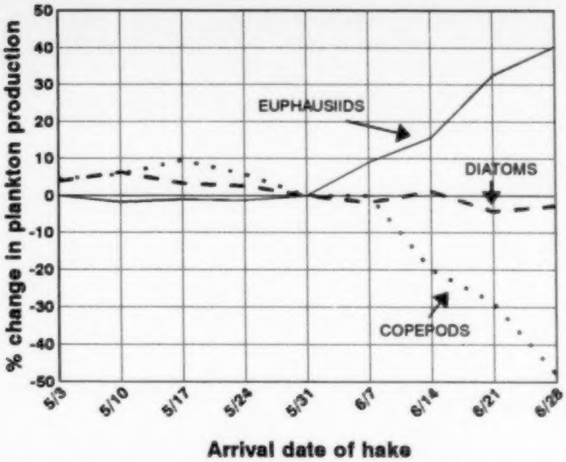

FIG. 7. Simulated effect of variable hake immigration timing into the Eddy region on annual plankton production. Changes in production are calculated as percent deviations from standard run: diatoms: 332 g C·m$^{-2}$·yr$^{-1}$; copepods: 24.3 g C·m$^{-2}$·yr$^{-1}$; euphausiids: 11.9 g C·m$^{-2}$·yr$^{-1}$.

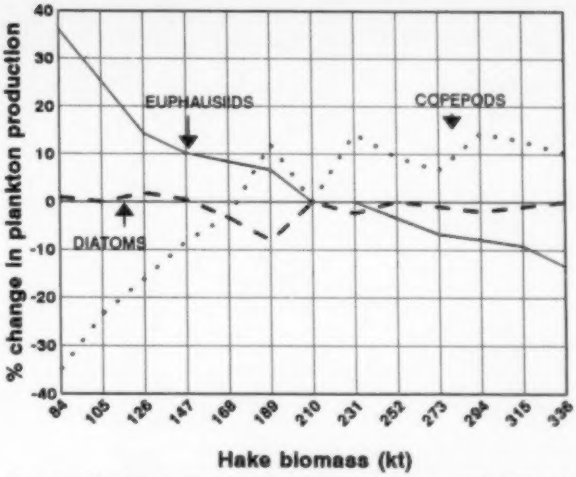

FIG. 8. Simulated effect of variable hake biomass (84–336 kt) on annual plankton production. Changes in production are calculated as percent deviations from standard run (see Fig. 7 legend).

plankton and fish than do perturbations in biological parameters (e.g., ingestion rates). In this study, we have also determined that there are two aspects of hake migration that impact plankton and resident fish production: the biomass and timing of arrival of hake.

*Hake arrival*

The arrival of hake on the southern B.C. shelf in summer varies from year to year. Migratory hake have been caught on the inner B.C. shelf as early as the first week in May and as late as the last week of June (B. Hargreaves, Department of Fisheries and Oceans, Nanaimo, B.C. unpublished data). In the simulations, we varied hake arrival times between these two possible boundaries on a weekly basis.

Simulated variability in hake arrival time causes significantly different euphausiid biomass patterns. The model predicts that if hake arrive in early May, euphausiid biomass only increases after hake begin to emigrate from the Eddy to the outer shelf in July–August. A late arrival of hake (mid-June) results in a "buildup" of euphausiid biomass during early June and causes an increase in annual euphausiid production of between 10 and 40% (Fig. 7) because of reduced predation pressure on euphausiids in early summer. On the other hand, late hake arrival negatively impacts copepod annual production indirectly via effects of euphausiid predation; an intermediate hake arrival date is most beneficial to copepod production. Hake arrival time does not significantly influence annual diatom production (Fig. 7).

*Hake biomass*

Variability in hake biomass also strongly impacts zooplankton production. We estimate variability in hake biomass using the temperature–biomass function discussed above (Ware and McFarlane 1994). The minimum and maximum average June–July water temperatures observed during the 1980s result in about 84 and 336 kt of hake entering the Eddy region, respectively. This represents a ±60% change in hake biomass from the standard run (210 kt).

Annual euphausiid production is negatively related to increases in hake biomass, while copepod production is positively related, and diatoms are relatively unaffected (Fig. 8). The magnitude of change in euphausiid production caused by hake biomass variability is asymmetrical. For example, hake biomass >210 kt results in a relatively narrow range of annual euphausiid production (a 0–10% decrease). Conversely, when hake biomass is <200 kt the range of euphausiid production is broad (0–35% increase; Fig. 8). This asymmetrical response in euphausiid production is undoubtedly due to interactions between the nonlinear hake emigration function and euphausiid biomass.

## System Properties

We now describe and discuss some general properties of the Juan de Fuca Eddy system between 1985 and 1989: (1) annual production, (2) production-to-mean biomass (*P/B*) ratios, (3) annual mass balance budget, and (4) production efficiency.

*Annual production*

Annual plankton and fish production is calculated by summing daily biomass growth increments before losses and then converting to carbon using conversion factors discussed in Appendix 1. Diatoms produce about 14 times more carbon annually than copepods and 28 times more than euphausiids, while copepods are twice as productive as euphausiids (Table 2). The simulated annual diatom production of 332 g C·m$^{-2}$·yr$^{-1}$ is similar to estimates made in southern regions of the Coastal Upwelling Domain (150–540 g C·m$^{-2}$·yr$^{-1}$; Smith and Eppley 1982; Chavez et al. 1991). Simulated copepod production is about 1.2–1.6 times less than, while euphausiid production is within the range of, estimates made for the more productive Benguela Current upwelling region (30–40 and 6–20 g C·m$^{-2}$·yr$^{-1}$, respectively; Hutchings et al. 1991). Note that the present model generates annual euphausiid production that is about twice as high as that estimated by Robinson et al. (1993). The difference is due to modification of the coupled model to

TABLE 2. Simulated annual plankton and fish production properties for the Eddy region during the period 1985–89. Transfer efficiency is calculated by dividing zooplankton or fish production by diatom production and multiplying by 100. Adult herring properties are calculated for April 1 to November 15, while hake properties are for June 1 to October 15. Cop, copepods; Euph, euphausiids.

| Property | Diatoms | Cop | Euph | Juvenile herring | Adult herring | Hake |
|---|---|---|---|---|---|---|
| Production ($P$; g $\mathrm{C{\cdot}m^{-2}{\cdot}yr^{-1}}$) | 332.0 | 24.3 | 11.9 | 0.8 | 1.5 | 1.0 |
| Average biomas ($B$; g $\mathrm{C{\cdot}m^{-2}}$) | 2.7 | 0.9 | 1.5 | 1.6 | 2.4 | 5.0 |
| $P/B$ ratio | 123.0 | 27.0 | 7.9 | 0.5 | 0.6 | 0.2 |
| Transfer efficiency (%) | — | 7.3 | 3.6 | 0.2 | 0.4 | 0.3 |

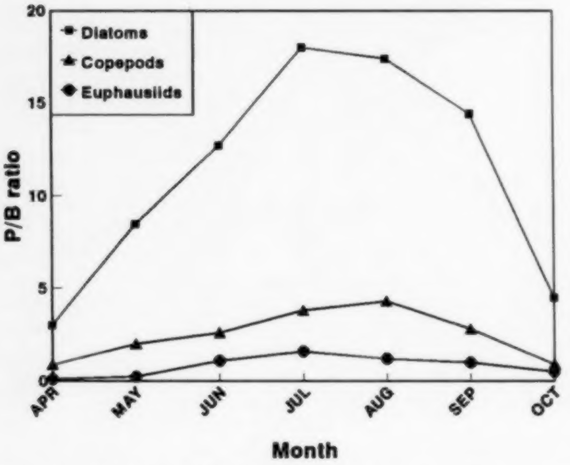

FIG. 9. Monthly estimates of production-to-mean biomass (*P/B*) ratios for diatoms, copepods, and euphausiids.

allow for (1) increased fish predation in late winter and early spring, (2) increased maximum and decreased minimum euphausiid gross growth efficiencies, and (3) increased import of euphausiid biomass.

Total annual simulated fish production is about100 times less than diatom production and about 11 times less than zooplankton production (Table 2). The simulated total fish production of 3.3 g $\mathrm{C{\cdot}m^{-2}{\cdot}yr^{-1}}$ is comparable with other productive shelf regions such as Georges Bank and the North Sea (2.0–4.0 g $\mathrm{C{\cdot}m^{-2}{\cdot}yr^{-1}}$; Jones 1984). Simulated total fish production is also about 1.7 times greater than Iverson's (1990) estimates for nonupwelling coastal and oceanic regions and about 5–8 times less than estimates for the very productive Peruvian upwelling system (17–27 g $\mathrm{C{\cdot}m^{-2}{\cdot}yr^{-1}}$; Jarre et al. 1990).

### *Production-to-mean biomass (P/B) ratios*

Given estimates of annual production, *P/B* ratios can be calculated. The simulated annual diatom *P/B* ratio is about 123:1, while for the upwelling seasonal the *P/B* ratio drops to 85:1 (due to higher average biomass). By comparison, annual phytoplankton *P/B* ratios range from about 114:1 to 153:1 in the southern Benguela upwelling system (Brown et al. 1991). Simulated monthly diatom *P/B* ratios during the upwelling season are about 10 times greater than in winter and about 2 times greater than in spring (Fig. 9). These results are consistent with expectations concerning the effects of greater light limitation on diatom growth in winter and limited nutrient supply in spring.

The simulated annual copepod *P/B* ratio (27) is consistent with estimates made for other upwelling regions (e.g., Benguela: 30–40$\cdot\mathrm{yr^{-1}}$; Hutchings et al. 1991). While the simulated euphausiid *P/B* ratio (8) initially seemed high, Robinson (1994) calculated an average maximum range of euphausiid *P/B* ratios of 5–7$\cdot\mathrm{yr^{-1}}$ from 11 published studies. For instance, Hutchings et al. (1991) estimated euphausiid production in the Benguela system to be 6–20 g $\mathrm{C{\cdot}m^{-2}{\cdot}yr^{-1}}$, with *P/B* ratios of 10–16$\cdot\mathrm{yr^{-1}}$. Thus, our simulated euphausiid *P/B* ratio is not unreasonable.

The simulated hake *P/B* ratio (0.2) is roughly 1.5–2.5 times less than estimates for hake in the more productive Peruvian upwelling system (Jarre et al. 1990) and the Benguela system (Shelton 1987). The calculated adult herring *P/B* ratio of 0.6 is about 2–4 times less than estimates for more productive clupeoids, such as the Peruvian anchovy or sardine ($P/B$ = 1.8–2.5; Jarre et al. 1990).

### *Annual mass balance budget*

A budget of the annual flux of biomass in the system during 1985–89 indicates that about 53% of diatom production (4929 $\mathrm{t{\cdot}km^{-2}}$) is transported from the surface layer of the Eddy to offshore regions (Fig. 10). The result that the largest fraction of diatom production is lost to export is consistent with observations that between 50 and 90% of the primary production is exported to slope regions in other coastal upwelling regions or else is recycled in the microbial loop (Walsh 1981: Ware 1992).

Model zooplankton graze about 40% of diatom production (Fig. 10), which is also consistent with estimates from the literature. For instance, between 5 and 50% (average 25%) of phytoplankton production is estimated to be graced by copepods in the southern Benguela depending upon season, state of upwelling, etc. (Verheye et al. 1992). Pillar et al. (1992) estimated that euphausiids only consume about 1–3% of primary production, about 5 times less than the simulated estimate. On the Washington midshelf, Landry and

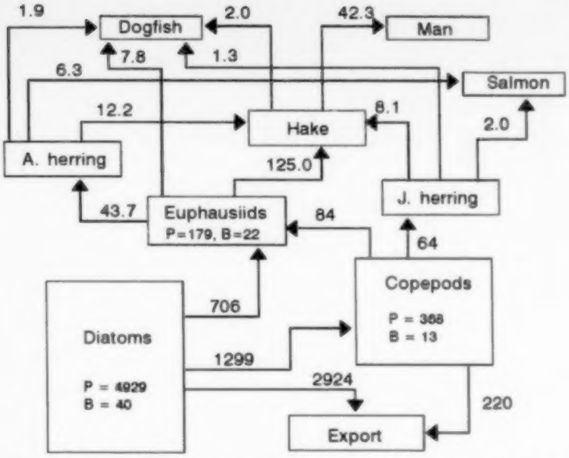

FIG. 10. Estimated average annual flux in the Juan de Fuca Eddy upwelling system between 1985 and 1989. Flows are in $t \cdot km^{-2} \cdot yr^{-1}$. Note that the sizes of the plankton boxes are proportional to the natural logarithm of their annual production (*P*, production; *B*, average annual biomass).

Lorenzen (1989) noted that copepods and euphausiids account for about 60% of the total zooplankton grazing impact, which removed about 20% of the primary production.

Roughly 23% of total copepod production is grazed by model euphausiids (Fig. 10). Pillar et al. (1992) similarly estimate that Benguela euphausiids consume between 21 and 35% of annual mesozooplankton production. The authors also noted that between 7 and 60% of copepod biomass can be removed by euphausiids depending upon the latter's "swarming" behaviour.

Model euphausiids lose 70, 25, and 5% of their annual production to hake, adult herring, and dogfish grazing, respectively (Fig. 10). Combined, the fish graze about 270 kt of euphausiids, with simulated consumption to mean biomass ratios (*C/B*) of about 1.7 for hake, 1.1 for adult herring, and 1.0 for dogfish. The simulated hake *C/B* is slightly less than estimates for Peruvian hake (1.8–2.2; Jarre et al. 1990). The herring *C/B* ratio is about 6 times less than that estimated for clupeoids off South Africa (6.9; Armstrong et al. 1991) and about 2 times less than estimates for Bering Sea herring (0.7; Laevastu et al. 1982). The comparison between simulated and observed fish *C/B* ratios must be made with caution, however, because most of the above studies used simplifying assumptions about seasonal variability in fish ingestion rates, diet composition, and biomasses.

### *Production efficiency*

Our annual mass balance budget for the Juan de Fuca Eddy upwelling system (Fig. 10) indicates that 11% of the annual primary production is converted into secondary (i.e., copepod and euphausiid) production, while about 1% of the annual primary production is converted into fish production. These estimates of production efficiency compare favourably with measurements for other systems. For example, 9–12% of the primary production in the Georges Bank – Gulf of Maine area is converted into secondary production, and 1.0–1.5% into fish (and squid) production (Sherman et al. 1988). By comparison, an estimated 0.3, 0.5, and 1.2% of the primary production in the southern California Current, Benguela, and Peruvian upwelling systems is converted into fish production (Ware 1992). The last two upwelling systems appear to be less efficient in converting primary production into fish production because most of the former seems to be exported offshore where it enters the mesopelagic food chain or deep-sea sediments, or else it is being recycled through the microbial loop and is therefore largely unavailable to support high trophic level carnivores. In part, because of its lower rate of export, the Juan de Fuca Eddy upwelling system appears to function almost as efficiently as the Peruvian upwelling system.

### Summary

We have developed a generic trophodynamics model to estimate the production potential and evaluate the functioning of the most abundant pelagic organisms on the continental shelf off southern Vancouver Island. The most important simulation result is that there is an imbalance between fish consumption and euphausiid production during the summer upwelling season. Our simulations indicate that a combination of high adult euphausiid growth and oceanographic mechanisms that import and accumulate euphausiids in the Eddy region is needed to satisfy the relatively high upper trophic level demand for euphausiids. Additional research is required to further understand and quantify these processes and their effect on the production dynamics of this system.

Our results also highlight the need to recognize that zooplankton production can be influenced by the migratory characteristics of pelagic fishes such as hake. The ability of our model to capture the seasonal dynamics in euphausiid biomass leads us to suggest that empirically derived functions, where predator migrations are linked to prey biomasses, be included when developing trophodynamic models of dynamic coastal regions.

The technique of simulation modelling has been shown to be an effective quantitative tool for evaluating the production properties of this coastal upwelling system. We have found that production estimates, *P/B* ratios, and transfer efficiencies are comparable with more productive coastal upwelling regions such as the Benguela Current. The high production potential of the plankton is a major reason why the continental shelf off southern Vancouver Island has been the most productive fishing region along the west coast of Canada for the past 70 yr.

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## Appendix 1

This appendix describes the mathematical equations used to represent the major processes affecting the biomass of diatoms ($D$), copepods ($C$), euphausiids ($E$), adult herring ($H$), juvenile herring ($J$), Pacific hake ($W$), spiny dogfish ($S$), and chinook salmon ($O$). The system of difference equations are integrated forward, from empirically derived initial conditions, by 1-d time intervals for 1 yr using the software package Biological SIMulation (BSIM; Silvert 1989). Integrations shorter than 1 d are not warranted because of additional assumptions and complexities required to represent short-term physiological and behavioural characteristics of the plankton.

Diatom biomass is modelled as milligrams of chlorophyll *a* per cubic metre (mg Chl a·m$^{-3}$), while zooplankton biomass is in grams dry weight per square metre (g DW·m$^{-2}$). Fish biomass is in metric tonnes of wet weight per square kilometre (1 t WW·km$^{-2}$ = 1 g WW·m$^{-2}$). Carbon is the common unit in the model. To convert chlorophyll *a* to carbon, we use a ratio ($C_c$) that varies from 35:1 to 50:1. It is well known that $C_c$ is lower when phytoplankton are growing vigorously compared with when populations are resource (nutrient/light) limited (Parsons et al. 1984). We assume that 15% of fish wet weight is dry weight ($W_d$), 45% of fish (and zooplankton) dry weight is carbon ($D_c$), and thus 6.75% of fish wet weight is carbon ($W_c$; Parsons et al. 1984). Refer to Table 1 for parameter values and to Robinson et al. (1993) for more discussion concerning the plankton dynamics of the coupled model.

Diatoms

$$D_{t+1} = D_t + \frac{\Delta D}{\Delta t}$$

$$\frac{\Delta D}{\Delta t} = \left( \frac{U_d NL}{(N + H_n)(L + H_l)} - R_d U_d \right) DC_c$$

$$- \left( \frac{S_d}{Z} \left( 1 - \frac{N}{H_n} \right) \right) DC_c - \left( \frac{I_c Q(D - P_t)}{H_c + (D - P_t)} \right) CD_c$$

$$- \left( \frac{I_e Q(D - P_t)}{H_e + (D - P_t)} \right) ED_c - T_d T_e DC_c$$

= growth − sinking − copepod predation − euphausiid predation − offshore export.

The first term of the diatom biomass ($D$) equation represents the temperature-dependent growth rate ($U_d$) limited by the effects of light ($L$) and nitrate ($N$) concentrations. Daily diatom respiration as a proportion of diatom production is also considered ($R_d$ = 5%). The second term represents diatom biomass lost to sinking where $S_d$ is the maximum sinking rate (1 m·d$^{-1}$), $Z$ is the depth of the mixed layer, $N$ is nitrogen in the mixed layer, and $H_n$ is the nitrogen half-saturation constant (1 mmol·m$^{-3}$). The third and fourth terms represent diatom loss to grazing by copepods ($C$) and euphausiids ($E$), respectively. $I_c$ and $I_e$ are zooplankton ingestion rates, $H_c$ and $H_e$ are prey half-saturation constants, and $P_t$ is a diatom threshold concentration (10 μg C·l$^{-1}$). The fifth term represents diatom loss to offshore export as a function of the Ekman transport ($T_e$).

Copepods

$$C_{t+1} = C_t + \frac{\Delta C}{\Delta t}$$

$$\frac{\Delta C}{\Delta t} = \left( \frac{G_c I_c Q(D - P_t)}{H_c + (D - P_t)} \right) CD_c - \left( \frac{I_e Q(C - P_t)}{H_e + (C - P_t)} \right) ED_c$$

$$- \left( \frac{I_j Q(C - P_t)}{H_j + (C - P_t)} \right) JW_c - T_c T_e CD_c$$

= growth − euphausiid predation − juvenile herring predation − offshore export.

The first term on the right-hand side of the copepod biomass ($C$) equation represents growth from ingesting diatoms where $G_c$ is the gross growth efficiency, $I_c$ is the ingestion rate, $Q$ is the effect of temperature on ingestion, and $H_c$ is the prey half-saturation constant. The second and third terms represent copepods being eaten by euphausiids and juvenile herring, respectively. The last term represents copepods being exported offshore via Ekman transport ($T_e$). The maximum export rate ($T_c$; 13%·d$^{-1}$) occurs during the week of greatest transport; export during the remaining weeks is proportional to the maximum rate.

Euphausiids

$$E_{t+1} = E_t + \frac{\Delta E}{\Delta t}$$

$$\frac{\Delta E}{\Delta t} = \left( \frac{G_e I_e Q(D + C - P_t)}{H_e + (D + C - P_t)} \right) ED_c$$

$$- \left( \frac{I_h Q(E - P_t)}{H_h + (E - P_t)} \right) HW_c - (I_w Q W_e) WW_c$$

$$- (I_s Q S_e) SW_c + T_e R_e D_c$$

= growth − adult herring predation − hake predation − dogfish predation + import.

The first term on the right-hand side of the euphausiid biomass ($E$) equation represents growth from ingesting diatoms and copepods. The second term represents predation loss to adult herring ($H$). The third and fourth terms represent predation loss to hake ($W$) and dogfish ($S$) where $W_e$ and $S_e$ are the proportion of euphausiids in the hake and dogfish diets, respectively, and $I_w$ and $I_s$ are ingestion rates. The last term represents the import ($R_e$) of euphausiids from surrounding oceanic regions due to Ekman transport ($T_e$).

Nitrogen

$$N_{t+1} = N_t + \frac{\Delta N}{\Delta t}$$

$$\frac{\Delta N}{\Delta t} = - \left( \frac{U_d NLDC_c}{(N + H_n)(L + H_L)} \right) C_n$$

$$+ \left( \frac{X_z I_z QZD_c\,(P - P_t)}{H_z + (P - P_t)} \right) C_n + \left( \frac{U}{Z} N_{30} T_e \right)$$

$$+ T_e\,(N_0 - N)$$

= − uptake + zooplankton excretion + upwelling + mixing.

The first term in the nitrogen ($N$) equation represents uptake by diatoms (see above). The second term represents the addition of nitrogen from zooplankton excretion where $X_z$ is the zooplankton excretion rate and $C_n$ is the carbon to nitrogen conversion (6:1). The third term represents the addition of nitrogen from upwelling where $U$ is the upwelling rate (4 m·d$^{-1}$), $Z$ is the depth of the mixed layer, $N_{30}$ is the nitrogen concentration at 30 m, and $T_e$ is the Ekman transport function. The last term represents nitrogen addition from wind mixing (turbulance) where $N_0$ is the starting (winter) nitrogen concentration.

Adult Herring

$$H_{t+1} = H_t + \frac{\Delta H}{\Delta t}$$

$$\frac{\Delta H}{\Delta t} = \left( \frac{G_h I_h Q(E - P_t)}{H_h + (E - P_t)} \right) H - (I_w Q(1 - W_e) W_h W)$$

$$- (I_s Q(1 - S_e) S_h S) - I_o O_h O + M_h$$

= growth − hake predation − dogfish predation − salmon predation ± migration.

The first term in the adult herring biomass ($H$) equation represents growth from ingestion of euphausiids where $G_h$ is the gross growth efficiency, $I_h$ is the ingestion rate, and $Q$ is the temperature effect on ingestion. The next three terms represent losses to fishes where $W_e$ and $S_e$ are the proportion of euphausiids in the hake ($H$) and dogfish ($S$) diet and $W_h$, $S_h$, and $O_h$ represent the fraction of the hake, dogfish, and salmon ($O$) herring ration that consists of adult herring. The last term represents the immigration and emigration of adult herring ($M_h$).

Juvenile Herring

$$J_{t+1} = J_t + \frac{\Delta J}{\Delta t}$$

$$\frac{\Delta J}{\Delta t} = \left( \frac{G_j I_j Q(C - P_t)}{H_j + (C - P_t)} \right) J - (I_w Q(1 - W_e) W_j W)$$

$$- (I_s Q(1 - S_e) S_j S) - I_o O_j O + M$$

= growth − hake predation − dogfish predation − salmon predation ± migration.

The equation describing juvenile herring dynamics ($J$) is the same as for the adult herring except juvenile herring ingest copepods.

Pacific Hake

$$W_{t+1} = W_t + \frac{\Delta W}{\Delta t}$$

$$\frac{\Delta W}{\Delta t} = (G_w I_w Q) W - (I_s Q S) S_w - F_w + M_w$$

= growth − dogfish predation − fishing loss ± migration.

The first term on the right-hand side of the hake biomass ($W$) equation represents growth from ingesting euphausiids and herring where $I_w$ is the ingestion rate of euphausiids and adult and juvenile herring. The next term represents the loss of hake to dogfish predation where $S_w$ represents the proportion of hake in the dogfish diet. The third term is the loss of hake to the summer fishery ($F_w$) and the last term represents the immigration and emigration of hake ($M_w$).

## Appendix 2

The observed euphausiid biomass pattern indicates that *T. spinifera* biomass is relatively low in May to July and increases to maximum concentrations from August to October (Fig. A1; see also Mackas 1992). Several independent lines of evidence suggest that this pattern may not be fully capturing the seasonal dynamics in euphausiid biomass. Specifically, early summer euphausiid biomass may have been underestimated because of (1) known biases associated with sampling euphausiids during the day, (2) data describing Pacific hake ingestion, and (3) biomass from expected euphausiid population size structure during spring. The remainder of this appendix presents an overview of the data supporting a revised euphausiid biomass pattern. This overview is not definitive but rather synthesizes available information and highlights potential underestimates of euphausiid biomass in spring and early summer.

Night/Day Catch Differences

Evidence that euphausiid biomass in the Eddy region is underestimated comes from the fact that the majority of early summer samples were colleced during daylight, while late-summer and fall samples were collected primarily during the night (Fig. A1). The time of collection strongly influences euphausiid biomass estimates because of the animal's vertical diel migratory behaviour. Generally, larger stages of most euphausiid species reside in relatively deep (>100 m) water during the day. The daytime depth is influenced by light levels, water temperature, oxygen concentration, and other factors (Anderson and Nival 1991). At night, euphausiids are found primarily in near-surface waters. The animals vertically migrate to/from day and night depths during crepuscular periods.

Given the vertical migratory behaviour of euphausiids, one would expect night collections to contain more biomass than day collections. Perusal of the literature indicates that night samples can maximally yield, on average, between 15 and 28 times more euphausiid biomass than corresponding day samples (Table A1). The variability in night/day catch ratios among studies is likely due to inherent patchiness in abundance, seasonal and interannual effects, and different species and sizes of euphausiids. However, it is generally accepted that higher nighttime biomass collections are due to increased availability of euphausiids and/or to less visual avoidance of the sampling gear (see next section). Everson and Bone (1986), for example, noted that *E. superba*'s ability to detect an oncoming net was hampered by darkness.

To evaluate night/day catch effects on euphausiid biomass estimates, we analyzed zooplankton samples collected from Eddy stations (C1, C2, C3, B8) and six shelf stations (<100 m) of the La Perouse region (see text, Fig. 1). The data were sorted into samples collected during the night and those collected during the day. Night is defined as the period 1 h after sunset to 1 h before sunrise, while day is 1 h after sunrise to 1 h before sunset; sunrise and sunset

TABLE A1. Observed maximum and average differences in night versus day catches of euphausiids using various sampling gear. In each study, night biomass estimates of euphausiids exceed day estimates by the amount indicated. Gear abbreviations: BN, bongo net; PN, plankton net; PL, plummet net; MN, MOCHNESS; BI, BIONESS. Maximum euphausiid lengths: *Euphausia pacifica* 25 mm; *Nematoscelis megalops* 25 mm; *Thysanoessa longipes* 30 mm; *T. gregaria* 30 mm; *Meganyctiphanes norvegica* 31 mm; *T. spinifera* 34 mm. N, night; D, day; ×, times. WCVI = west coast of Vancouver Island.

| Species | Gear | Catch ratio | Reference |
|---|---|---|---|
| *E. pacifica* | BN | N 10× > D | Youngbluth 1976 |
| *E. pacifica* | PN | N 20× > D | Brinton and Townsend 1981 |
| | BN | N 20× > D | |
| *E. pacifica* (Strait of Georgia) | BN | N 20× > D | Fulton et al. 1982 |
| *E. pacifica* (WCVI) | BN | N 10× > D | |
| *E. pacifica* (October) | PL | N 31× > D<br>(avg. 11×) | Hovekamp 1989 |
| *E. pacifica* (February) | PL | N 64× > D<br>(avg. 30×) | |
| *N. megalops* | MN | N 63× > D<br>(avg. 15×) | Wiebe et al. 1982 |
| *T. longipes* | BN | N 6× > D | Fulton et al. 1982 |
| *M. norvegica* | BI | N 14× > D<br>(avg. 7×) | Cochrane et al. 1991 |
| *T. gregaria* | PN | N 50× > D<br>(avg. 23×) | Brinton 1967 |
| Average (maximum values) | | N 28× > D | |
| Average (average values) | | N 15× > D | |
| *T. spinifera* | BN | N 20× > D | This study |

were determined from the *Nautical Atlas* for 50°N. Data falling within the two crepuscular periods were excluded from all analyses. This protocol prevents potential bias of overestimating biomass as a result of vertically migrating euphausiids (Alton and Blackburn 1972).

Average and standard deviations were calculated for night and day catches for the Eddy and shelf regions. The results indicate that *T. spinifera* biomass is about 20–30 times higher when sampled at night than during the day (Table A2). Inclusion of crepuscular data reduces the night/day catch ratios by 30–40%, suggesting that sampling around sunrise or sunset is more efficient in capturing vertically migrating euphausiids.

A simple test of the validity of the derived first-order night/day catch ratio for euphausiids involves comparing it with copepod data. We expected a priori that copepod samples should contain similar biomass estimates irrespective of time of collection. This is expected because most copepod stages and species reside within the upper 50 m of the water column during day and night and thus are equally available to the sampling gear (Mackas 1992). As expected, there are marginal differences between day and night estimates of copepod biomass (Table A2).

A comparison of *T. spinifera*'s night/day catches with the other abundant euphausiid *E. pacifica* indicates that the former species has a roughly 10 times higher night/day ratio (Table A2). The difference in night/day catch ratios between the two euphausiid species is consistent with what has been hypothesized about *T. spinifera*'s feeding behaviour. During the day, *T. spinifera* may feed on detrital matter on, or very near, the sea floor (D. Mackas, Department of Fisheries and Oceans, Sidney, B.C., personal communication; see also Cochrane et al. 1991). Thus, *T. spinifera* is

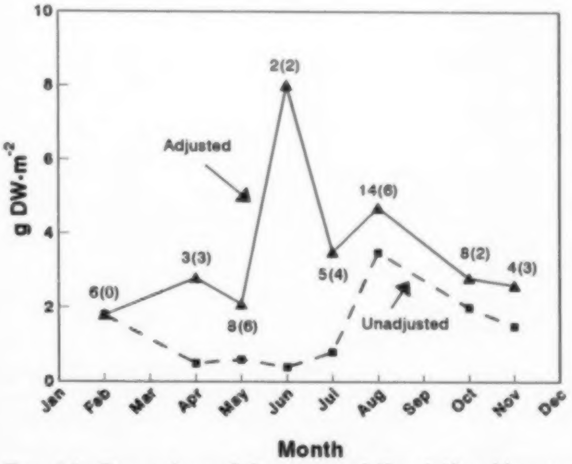

FIG. A1. Comparison of the measured *T. spinifera* biomass pattern (broken line) with the pattern obtained after adjusting day samples by 20 times (solid line). Nighttime and crepuscular samples were not adjusted. Lines join arithmetic averages. Note that 23 of 50 samples were collected during the day. Refer to Fig. 4.

TABLE A2. Estimates of zooplankton biomass (mg DW·m$^{-3}$) collected by bongo nets in the Eddy and shelf stations of the La Perouse region during night and day (AV. average; SD. standard deviation; *n*. number of samples; N/D, night/day ratio).

| | | Eddy stations | | | Shelf stations | | |
|---|---|---|---|---|---|---|---|
| | | *N* | D | N/D | *N* | D | N/D |
| *T. spinifera* | AV | 35.3 | 1.7 | 21 | 18.4 | 0.6 | 30 |
| | SD | 48.0 | 1.7 | | 57.0 | 0.6 | |
| | *n* | 19 | 22 | | 17 | 9 | |
| *E. pacifica* | AV | 3.8 | 1.5 | 2.5 | 2.1 | 0.9 | 2.4 |
| | SD | 8.3 | 2.1 | | 4.3 | 2.1 | |
| | *n* | 19 | 23 | | 9 | 8 | |
| Copepods | AV | 4.6 | 15.0 | 0.3 | 14.0 | 20.6 | 0.7 |
| | SD | 5.4 | 18.7 | | 16.1 | 24.7 | |
| | *n* | 22 | 41 | | 33 | 35.0 | |

less available to "pelagic" samplers (bongo nets) during the day than at night when it feeds in near-surface waters. Apparently, the omnivorous *E. pacifica* maintains itself in the water column and is thus more susceptible to sampling with bongo nets day or night. Ultimately, a combination of lower availability to nets and greater net avoidance by the larger *T. spinifera* is likely responsible for its 10 times greater night/day catch ratio.

## Hake Ingestion

Additional evidence that euphausiid biomass is underestimated in the Eddy in early summer is derived indirectly from consumption of euphausiids by dominant pelagic fish such as Pacific hake. We assume that the pelagic fish are sampling (eating) euphausiids in proportion to the latter's availability. This assumption is not unreasonable for hake, herring, and/or dogfish given that euphausiids make up large fractions (>60–70%) of their diets (Tanasichuk et al. 1991). The ingestion rate of hake and the fraction of euphausiids in the hake diet both suggest that there is a relatively high biomass of euphausiids in early summer and substantially fewer euphausiids in late summer and early fall (see text, Fig. 6). This seasonal trend is opposite that observed in data derived from sampling euphausiids with bongo nets.

The discrepancy between euphausiid biomass patterns predicted from fish consumption estimates and from net sampling may be related to the fact that each "method" is sampling a different portion of the euphausiid population. Tanasichuk et al. (1991), for example, noted that the majority of euphausiids making up fish diets are larger adults (>20 mm). Large euphausiids may not be effectively sampled by bongo nets because the animals avoid the small net mouth opening or avoid the vibration from the net bridle, among other factors (Wiebe et al. 1982). Data in Brinton and Townsend (1981) also indicate that oblique tows of bongo nets did not capture *T. spinifera* >21 mm; the net most efficiently sampled individuals <17 mm. Other studies have also noted differences in euphausiid population size structure when comparing net catches and euphausiid predator diets. Daly and Macaulay (1988) captured "small" *E. superba* (<25 mm) with a plummet net but generally "missed" the majority of biomass of large euphausiids (>25 mm). The authors also noted that seal and seabird stomachs consisted primarily of larger euphausiids (35–55 mm), a situation perhaps not unlike the Eddy region in early summer (see also Cochrane et al. 1991).

## Euphausiid Population Size Structure

Final support for the idea that euphausiids, particularly larger adults, are undersampled during early summer is based on expected population size structure. Euphausiid populations in the Eddy region in early summer consist primarily of older, larger adults (>1 yr and 20 mm) and a mode of younger, smaller individuals (<1 yr and 10 mm; Simard and Mackas 1989). By late summer or early fall, the euphausiid population consists primarily of smaller, younger animals; larger, older animals decline in abundance because of natural mortality, particularly predation. Day (1971) similarly observed on the southern B.C. shelf that lower euphausiid biomass in November, compared with higher biomass in May, was due to the absence of larger *T. spinifera* (and *E. pacifica*). More recent sampling of *T. spinifera* populations in nearby Barkley Sound also indicates a bimodal pattern in euphausiid biomass, with peaks in late spring and late fall (R. Tanasichuk, Department of Fisheries and Oceans, Nanaimo, B.C.).

The empirical euphausiid biomass estimates in late spring/early summer are thus consistent with what is expected from net avoidance bias and euphausiid population size structure. That is, net derived euphausiid biomass estimates are low in early summer because juvenile biomass is relatively low; late summer/fall biomass estimates are high because more susceptible stages of euphausiids are available to the bongo net. Cochrane et al. (1991) also noted for the Scotian shelf "...a consistent pattern characterized by a greater proportion of smaller, more juvenile euphausiids in the fall season. The comparatively low June catches may be partly due to more efficient net avoidance by larger adult euphausiids."

## Adjusted Euphausiid Biomass Pattern

In summary, within the Eddy region during the late spring/early summer, it is apparent that (1) net sampling for large euphausiids was conducted with an inefficient, small bongo net primarily during daylight, (2) pelagic fish feeding data suggest high euphausiid biomass, and (3) *T. spinifera* populations consist primarily of large adults.

We conclude that it is not unreasonable to assume that *T. spinifera* biomass was underestimated by as much as 20 times during this period (Table A2). To generate a euphausiid seasonal biomass curve that is consistent with the above evidence, we apply a first-order night/day catch ratio of 20 times to all day samples of *T. spinifera*; all crepuscular and nighttime data are left unadjusted. This approach assumes that nighttime net sampling is 100% efficient at catching euphausiids; undoubtedly, however, there is even some net avoidance at night. The adjusted seasonal biomass pattern differs from the unadjusted biomass pattern mainly in that the estimated euphausiid biomass in late spring/early summer (April–July) is substantially higher (Fig. A1). This results from correcting a large number of daytime samples collected during this period.

# Rapid Growth, High Feeding Rates, and Early Piscivory in Young-of-the-Year Bluefish (*Pomatomus saltatrix*)

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Juanes, F., and D.O. Conover. 1994. Rapid growth, high feeding rates, and early piscivory in young-of-the-year bluefish (*Pomatomus saltatrix*). Can. J. Fish. Aquat. Sci. 51: 1752–1761.

The bluefish (*Pomatomus saltatrix*) is an offshore-spawning estuarine-dependent fish that is abundant along the east coast of North America. Young-of-the-year bluefish undergo a habitat shift from offshore waters to inshore nursery areas at about 40–70 mm total length which coincides with an increased growth rate and a diet shift from planktivory to piscivory. Here, we measure growth of young-of-the-year bluefish on different diets and estimate consumption rates both in the field and in the laboratory. Growth on a fish diet is significantly higher than that on a zooplanktivorous diet. Bluefish have among the highest evacuation (5–7 h), consumption (20–30% body weight/d), and specific growth rates (1–2 mm/d) reported for temperate fishes and are similar to those for pelagic tropical species. These results suggest that bluefish may exhibit a tropical feeding physiology in temperate estuarine nurseries as a way to achieve rapid growth rates.

Le tassergal (*Pomatomus saltatrix*) est un poisson estuarien frayant au large qui est abondant le long de la côte est de l'Amérique du Nord. Le jeune de l'année quitte son habitat au large pour gagner les viviers le long de la côte lorsqu'il atteint environ 40–70 mm de longueur totale, ce qui coïncide avec une augmentation du taux de croissance et une modification du régime alimentaire, de planctivore à piscivore. Ici, on mesure la croissance du jeune tassergal sur différents régimes, et on évalue la consommation dans la nature et au laboratoire. La croissance sur un régime à base de poisson est significativement plus rapide que sur un régime zooplanctivore. Les taux d'évacuation (5–7 h), de consommation (20–30% du poids corporel/d) et de croissance spécifique (1–2 mm/d) du tassergal sont parmi les plus élevés des poissons vivant en eau tempérée et sont semblables à ceux que l'on observe chez les espèces tropicales pélagiques. Ces résultats indiquent que le tassergal peut manifester un comportement alimentaire tropical dans des viviers estuariens tempérés de manière à croître plus rapidement.

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The bluefish (*Pomatomus saltatrix*) is a highly migratory species with a worldwide distribution (Le Gall 1934: Briggs 1960; Champagnat 1983). Along the east coast of North America, bluefish appear to have two distinct major spawning seasons and locations: a spring spawning in the South Atlantic Bight and a summer spawning in the Middle Atlantic Bight (Norcross et al. 1974; Kendall and Walford 1979; Chiarella and Conover 1990). Spring-spawned fish are advected northwards along the edges of the Gulf Stream and move into estuaries of the Middle Atlantic Bight in May and June. Summer-spawned fish recruit to these same estuaries in mid- to late August (Nyman and Conover 1988; Shima 1990; McBride and Conover 1991). Young-of-the-year (YOY) bluefish undergo the habitat shift from offshore waters to inshore nursery areas at about 40–70 mm total length (TL) (Nyman and Conover 1988; McBride and Conover 1991; Juanes 1992). This habitat shift coincides with a diet shift from planktivory to piscivory (Marks and Conover 1993) and with an increase in growth rate (McBride and Conover 1991). These estuarine growth rates are unusually high for a temperate inshore fish (1.2–1.8 mm/d, Nyman and Conover 1988; McBride and Conover 1991) and allow YOY bluefish to attain a larger size at age 1 than any other morphologically similar species in this area (Nyman and Conover 1988; Chiarella and Conover 1990).

The primary goal of this study was to examine how the rapid growth of YOY bluefish is achieved. First, we asked whether bluefish may increase their growth rate as a consequence of the diet shift to piscivory. We tested this hypothesis by determining if growth on a fish diet is greater than growth on an invertebrate diet. Second, we explored the physiological bases for this rapid growth. The distribution of bluefish is generally restricted to waters warmer than 15°C, suggesting that it is a warmwater species and may exhibit physiological traits more characteristic of tropical than temperate fishes (Le Gall 1934; van der Elst 1976). Tropical fishes are characterized by relatively high digestion and consumption rates (Fange and Grove 1979; Pandian and Vivekanandan 1985; Pauly 1989). Hence, the rapid growth of YOY bluefish may be a consequence of high rates of feeding and digestion. We thus estimated consumption and gut evacuation rates in the laboratory and also assessed consumption rates in the field from diel variations in weight of stomach contents on two dates during the summer. These estimates were then compared with published results for temperate and tropical species.

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## Material and Methods

### Diel Diet and Gut Fullness

Samples of YOY bluefish were collected on June 28–29 and July 26–27, 1989. Collections were made every 4 h starting at 06:00 with three hauls of two 30-m seine nets at two adjacent beaches in Great South Bay (GSB) near Islip, Long Island, N.Y. (40°10′N, 73°10′W). All bluefish collected were immediately frozen on dry ice.

In the laboratory, bluefish were thawed, measured (±1.0 mm TL), and weighed (±0.01 g wet weight). Gut contents were weighed (±0.01 g), measured (±1.0 mm TL), and identified to the lowest possible taxon. Gut fullnesses were calculated as the wet weight of the gut contents divided by the wet weight of the eviscerated bluefish. Mean lengths of bluefish captured at each time were compared using ANOVA after testing for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Bartlett's test). If treatment effects were significant, mean lengths were further compared using a Tukey HSD multiple comparison test. To assess whether feeding increased through the day, correlations between time of day and gut fullness for daylight hours were evaluated using a Pearson correlation coefficient.

Sunrise and sunset occurred at about 05:30 and 20:30, respectively, on both sampling dates. The moon was in its last quarter on both dates, although the moonrise occurred later during the first sampling period (on June 29 at 01:30, and on July 26 at 23:57). Water temperatures were measured at both sites before each series of net hauls using a thermometer. Mean water temperature was significantly higher on July 26–27 (27.67 ± 1.21°C (SD)) than on June 28–29 (25.83 ± 0.82°C) ($t = 0.075$, df = 10, $p = 0.0117$).

### Evacuation Rates

Three gut evacuation experiments were conducted using different-sized bluefish and the Atlantic silverside (*Menidia menidia*) as prey. Small bluefish were used on September 11, 1990 (mean bluefish TL = 74.20 mm, range = 57–96, mean weight = 3.207 g, $n = 10$; prey mean wet weight = 0.127 g; mean prey weight/predator weight = 3.972%). Medium bluefish were tested on August 6, 1990 (mean TL = 133.83 mm, range = 121–143, mean wet weight = 19.85 g, $n = 17$; prey mean wet weight = 0.279 g; mean prey weight/predator weight = 1.407%). Large bluefish were used on September 2, 1988 (mean TL = 154.82 mm, range = 128–199, mean wet weight = 31.53 g, $n = 11$; prey mean wet weight = 1.08 g; mean prey weight/predator weight = 3.425%). Bluefish were placed in individual tanks 2 d before the experiment and deprived of food in order to standardize hunger levels. Individual bluefish were then fed one silverside of a predetermined size. Time of ingestion was noted. Bluefish were then chosen randomly at 30- or 60-min intervals. Their stomach contents were removed, measured, weighed, and expressed as percentage of prey weight remaining. A similar experiment using sand shrimp as prey (*Crangon septemspinosa*, mean wet weight = 0.522 g, mean prey weight/predator weight = 3.265%) was conducted on medium bluefish (mean TL = 125.8 mm, range = 110–142, mean wet weight = 15.98, $n = 10$) on August 3, 1990. All these experiments were conducted at temperatures ranging from 21 to 22°C.

Evacuation rates were estimated by computing regressions between percent prey remaining in the gut and time after ingestion using linear, exponential, and square root models. The relationship between percent prey remaining and time after ingestion for the three Atlantic silverside digestion experiments were compared using an ANCOVA after testing for homogeneity of slopes.

### Field Consumption Rates

Daily rations are commonly measured as functions of gut evacuation rates determined in laboratory studies and diel variations of stomach contents of field-collected fish. Because none of the available models for estimating daily ration (Jobling 1986; Persson 1986; Adams and Breck 1990; Wootton 1990) appeared to be particularly applicable to a warmwater piscivore eating relatively small prey, we used the four most appropriate and widely used models:

Eggers (1979) model:

$$C_{\Delta 24} = 24 \cdot r_e \cdot W_{\Delta 24}$$

Elliott and Persson (1978) model:

$$C_{\Delta t} = \frac{(\overline{W}_t - \overline{W}_{t-1} \cdot e^{-r_e t}) \cdot r_e t}{1 - e^{-r_e t}}$$

Pennington (1985) model:

$$C_{\Delta t} = 2 \cdot r_{0.5} \cdot \sum_{i=1}^{n} \left( \frac{\sqrt{W_i}}{n} \right) + \frac{(\overline{W}_t - \overline{W}_{(t-1)}}{t-(t-1)}$$

Olson and Boggs (1986) model:

$$\hat{r} = \left( \sum_{i=0}^{l} \frac{\overline{W}_i}{A_i} \right) \cdot 24$$

where $C_{\Delta t}$ is food consumption between sampling periods at time $t$ and $t - 1$, $W_t$ is average prey weight at time $t$, $W_i$ is the prey weight in an individual bluefish at time $t$, $n$ is the number of bluefish, $r_e$ is the exponential evacuation rate, $r_{0.5}$ is the square root evacuation rate, $A_i$ is the integral of the evacuation function, $W_i$ is the mean stomach contents per predator, $l$ is the number of prey types, and $\hat{r}$ is the feeding rate (grams per hour). Daily meal is calculated by multiplying $\hat{r}$ by 24 h, and daily ration is the daily meal expressed as a percent of body weight. The Eggers (1979) and Elliott and Persson (1978) models both assume exponential gastric evacuation. The Pennington (1985) model is usually used with a square root evacuation model, although this model can accommodate any form of the evacuation function. The Olson and Boggs (1986) model also does not require any specific form of the gastric evacuation function, but has commonly been used with a linear evacuation rate. Because fish were collected over a 20-h period (06:00–02:00), for those models that require 24-h sampling (Elliott and Persson and Pennington), we assumed the following 06:00 gut fullness to be similar to the previous 06:00 gut fullness.

### Consumption, Growth and Gross Efficiency in the Laboratory

A 20-d-long experiment was conducted in 1990 to estimate short-term growth rates, mean feeding rates, and gross growth efficiency on four natural diets. The experiments were conducted with four groups of three bluefish in separate tanks (75 × 75 × 30 cm). Each group was randomly selected to

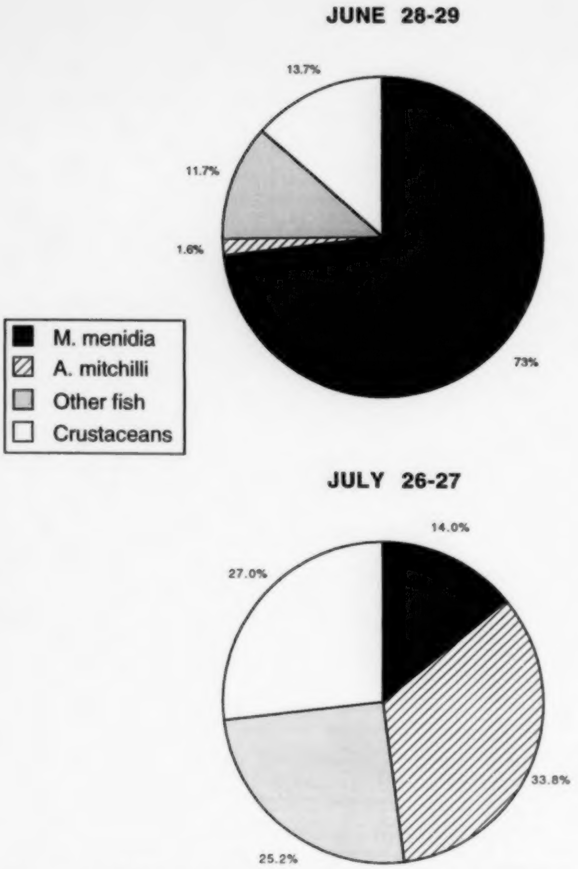

FIG. 1. Diet description by weight of the June 28–29 and July 26–27 samples of YOY bluefish. "Crustaceans" category includes grass and sand shrimp (*Palaemonetes vulgaris* and *Crangon septemspinosa*, respectively), zoea, and unidentified shrimp remains. "Other fish" category includes rainwater killifish (*Lucania parva*), northern puffer (*Sphoeroides maculatus*), permit (*Trachinotus falcatus*), winter flounder (*Pleuronectes americanus*), unknown fish species, and unidentified fish remains.

be fed one of four live diets: adult brine shrimp (*Artemia* sp.), sand shrimp, and small (20–40 mm TL) or medium (50–70 mm TL) Atlantic silverside. Fish were kept under a constant light regime (14 h light : 10 h dark) and temperature (range = 20–23°C, mean = 21.45°C (SD = 1.13)). All bluefish were anaesthetized with MS-222, weighed, and measured before they were put into tanks. Individuals within each tank were marked by clipping small portions of the caudal fin. Members of each tank were selected to be as similarly sized (within 1–3 mm) as possible. All bluefish were starved for 24 h before being fed for the first time. Live shrimp and Atlantic silverside were added after measuring, weighing, and counting. *Artemia* were blotted dry before weighing. Enough food was added to exceed daily ration requirements (calculated in previous experiments). On each successive day, remaining shrimp and Atlantic silverside prey were counted, and enough additional prey individuals were added to again exceed calculated daily rations. Dead prey were collected from the bottom of the tank, weighed, and subtracted from the previous day's consumption total. Tanks were checked daily for potential bluefish mortality. All bluefish survived the period of the experiment and prey were never totally consumed within any treatment. After 19 d, all prey were removed, and bluefish were starved for 24 h, before weighing and measuring.

Daily specific growth rate ($G$) of each fish was calculated as

$$G = \left[ \frac{\ln \mathrm{Wt}_f - \ln \mathrm{Wt}_i}{d} \right] \cdot 100$$

where $\mathrm{Wt}_f$ = final wet or dry weight, $\mathrm{Wt}_i$ = initial wet or dry (calculated from the wet weight/dry weight regression, see below) weight, and $d$ = number of feeding days (=18).

Daily specific feeding rates were calculated by dividing the overall amount of food consumed within each tank by the average size of bluefish during the experiment in each tank (calculated as $(\ln \mathrm{Wt}_f + \ln \mathrm{Wt}_i)/2$). Feeding rates were expressed in terms of wet weight (grams per gram), dry weight (grams per gram), and energy (kilojoules per kilojoule, calculated from dry weights, see below). Gross growth efficiency ($K$) was calculated as the total fish weight gain per prey weight consumed. The mean daily number of prey consumed per tank was calculated from daily counts of remaining Atlantic silverside and shrimp. Daily numbers of *Artemia* ingested were calculated as the total consumption divided by the average weight of an individual.

Mean daily specific growth rates, feeding rates, growth efficiencies, and numbers of prey items consumed (three tanks or replicates per diet treatment) were compared among diet treatments using ANOVA after testing for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Bartlett's test). If treatment effects were significant, means were further compared using a Tukey HSD multiple comparison test.

A range of Atlantic silverside, shrimp, and bluefish sizes were dried in an oven (at 60°C) until a constant weight was achieved (usually 24 h). The regressions relating wet weight (WW, grams) to TL (millimetres) and dry weight (DW, grams) to wet weight were as follows:

Bluefish:

$$\mathrm{DW} = 0.211 \times \mathrm{WW}^{1.039},\ n = 54,\ r^2 = 0.999,\ p < 0.0001$$

$$\mathrm{WW} = (1.483 \times 10^{-6}) \times \mathrm{TL}^{3.347},\ n = 54,\ r^2 = 0.996,\ p < 0.0001$$

Shrimp:

$$\mathrm{DW} = 0.247 \times \mathrm{WW}^{0.969},\ n = 21,\ r^2 = 0.990,\ p < 0.0001$$

$$\mathrm{WW} = (8.685 \times 10^{-6}) \times \mathrm{TL}^{3.00},\ n = 21,\ r^2 = 0.984,\ p < 0.0001$$

Atlantic silverside:

$$\mathrm{DW} = 0.250 \times \mathrm{WW}^{1.016},\ n = 55,\ r^2 = 0.995,\ p < 0.0001$$

$$\mathrm{WW} = (4.303 \times 10^{-6}) \times \mathrm{TL}^{3.034},\ n = 55,\ r^2 = 0.994,\ p < 0.0001.$$

Five 1-g samples of *Artemia* were also dried for 24 h to obtain a dry weight to wet weight conversion:

$$1\ \mathrm{g\ WW} = 0.065 \pm 0.0007\ (\mathrm{SD})\ \mathrm{g\ DW},\ n = 5.$$

In addition, four sets of 50, 40, 30, and 20 individuals were dried to obtain the average dry weight of an individual *Artemia* (=0.00338 ± 0.000162 (SE) g ).

Estimates of the caloric value of Atlantic silverside (21.2 kJ/g dry weight), sand shrimp (16.2 kJ/g dry weight), and bluefish (20.8 kJ/g dry weight) were obtained from Steimle and Terranova (1985). The caloric content of *Artemia* (24.5 kJ/g dry weight) was obtained from Leger et al. (1986).

## Results

### Diet and Gut Fullness

A total of 256 YOY bluefish were collected during the two sampling periods. Because both of the collection dates took place before any summer-spawned bluefish appeared (Juanes 1992), only spring-spawned bluefish were sampled. On both dates, bluefish diets consisted mainly of fish (86.3% on June 28–29 and 73.0% on July 26–27) although composed of different prey species (Fig. 1). The first diel sample occurred early in the estuarine phase, when bluefish were still relatively small (mean bluefish TL = 98.06 mm, mean wet weight = 9.357 g, Fig. 2) and when their diet was dominated by Atlantic silverside (73.04% by weight, Fig. 1). The percentage of fish by weight in the diet remained fairly constant through the sampling period and the crustacean part of the diet was composed mainly of crab zoea (see Juanes 1992). On July 26–27, bluefish were significantly larger (mean TL = 135.45 mm, mean wet weight = 26.08 g; $t = 7.450$, df = 86, $p < 0.001$), having grown about 1.37 mm/d (and 0.62 g/d) between sampling dates. On this second sampling date, the diet was composed mainly of bay anchovy (*Anchoa mitchilli*) (34%), shrimp (21%), and Atlantic silverside (14%) (Fig. 1). The percentage of fish by weight in the diet was fairly constant throughout the sampling period except for the 10:00 sample when 85% of the diet was composed of the two species of shrimp (Juanes 1992). Although bluefish were significantly larger on July 26–27, mean teleost prey size was similar on both dates (June 28–29: mean prey TL = 28.17 ± 1.91 (SE) mm, $n = 35$; July 26–27: mean prey TL = 30.06 ± 1.74, $n = 53$; $t = 0.7139$, df = 86, $p = 0.477$).

On June 28–29, gut fullness increased through the day, peaked at 18:00, and then decreased through the night (Fig. 2). This pattern was mirrored by the percentage of empty guts at each time period: low during the day (≈20%) and higher at night (≈60%). There was a positive significant correlation between fullness and time of day for daylight hours ($r = 0.184$, $p = 0.044$), and similarly sized bluefish were collected at each time period (ANOVA $p = 0.166$). Gut fullness also increased during the daylight hours on July 26–27 ($r = 0.365$, $p = 0.002$). However, a second peak was observed at the 02:00 sample (Fig. 2). On this date, the percentage of empty guts was generally lower (≈10%) throughout the day. In addition, the two night samples (22:00 and 02:00) were composed of significantly larger bluefish than those collected during the daylight hours (ANOVA $p = 0.007$, followed by a Tukey HSD multiple comparison; Fig. 2).

### Evacuation Rates

Evacuation rates of YOY bluefish were uniformly high. Time to 90% digestion of prey for the three bluefish body sizes varied from 5.5 to 7 h. Both the slopes and the elevations of the regressions between percent prey remaining and time after ingestion for the three *Menidia* digestion experiments were statistically indistinguishable (slopes: $F = 0.454$, df = 34, $p > 0.05$; intercepts: $F = 2.344$, df = 36, $p > 0.05$; Fig. 3). The data for these three experiments were thus combined for further analyses. These combined data were then used to calculate the three types of evacuation rates, linear, exponential, and square root:

Linear:

$$(\%\text{rem}) = 67.933-9.535t,\ r^2 = 0.497,\ p < 0.0001,\ n = 39$$

Square root:

$$\sqrt{(\%\text{rem})} = 8.416-0.812t,\ r^2 = 0.492,\ p < 0.0001,\ n = 39$$

Exponential:

$$\text{l-n}\ (\%\text{rem}) = 4.372-0.306t,\ r^2 = 0.444,\ p < 0.0001,\ n = 39.$$

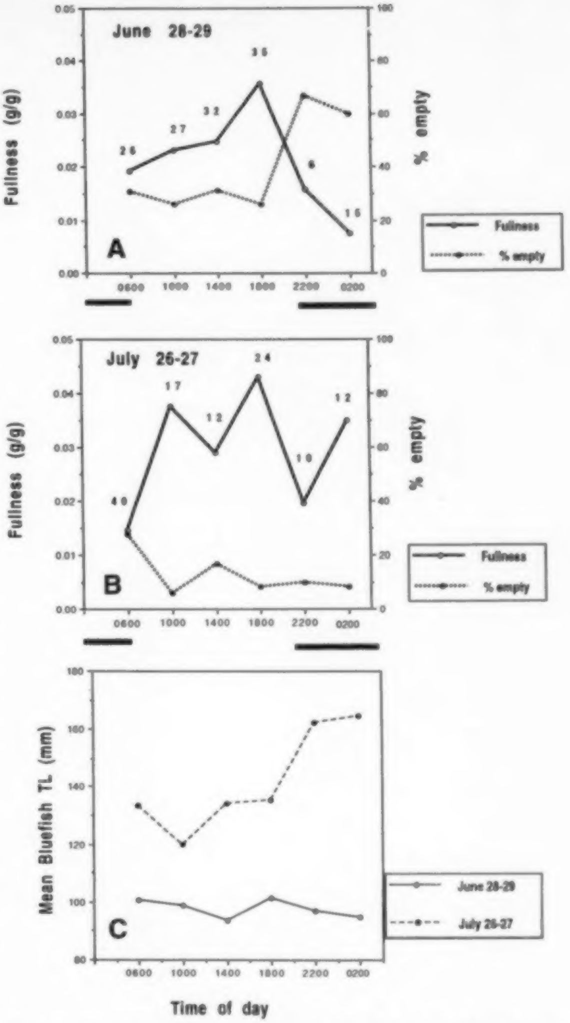

FIG. 2. (A and B) Diel gut fullness patterns and (C) mean bluefish sizes collected during June 28–29 and July 26–27, 1989. Dark bands represent periods of darkness. Numbers above points are sample sizes.

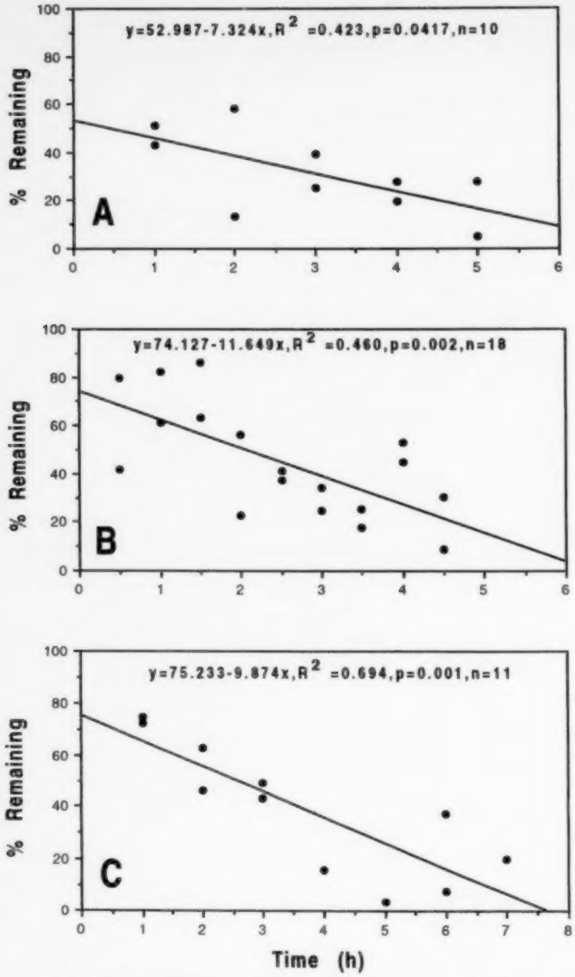

FIG. 3. Relationships between percent prey remaining and time for YOY bluefish feeding on Atlantic silverside. (A) September 11, 1990: mean bluefish TL = 74.20 mm, range = 57–96, mean weight = 3.207 g, *n* = 10; prey mean wet weight = 0.127 g; prey weight/predator weight = 3.972%; (B) August 6, 1990: mean TL = 133.83 mm, range = 121–143, mean wet weight = 19.85 g, *n* = 17; prey mean wet weight = 0.279 g; prey weight/predator weight = 1.407%; (C) September 2, 1988: mean TL = 154.82 mm, range = 128–199, mean wet weight = 31.53 g, *n* = 11; prey mean wet weight = 1.08 g; prey weight/predator weight = 3.425%.

The resulting (linear) regression equation for the shrimp digestion was (Fig. 4)

$$\%\text{rem} = 98.157 - 11.921t,\ r^2 = 0.606,\ p = 0.008,\ n = 10.$$

When Atlantic silverside and shrimp linear evacuation rates were compared, their slopes were statistically indistinguishable ($t = 0.6149$, df = 45, $p < 0.05$).

### Field Consumption Rates

The evacuation rate parameters were then incorporated into the four models for estimating daily ration in the field (i.e., linear, Olson and Boggs; square root, Pennington; exponential, Eggers and Elliott and Persson). The four models generated similar estimates of consumption: daily meal ranged from 1.57–3.07 g (16.78–32.88% body weight) on June 28–29 to 6.36–8.55 g (24.40–32.73% body weight) on July 26–27 (Table 1). The Olson and Boggs model incorporates prey-specific evacuation rates. When consumption was calculated using both shrimp and fish evacuation rates and incorporated the different amounts of these items consumed on the respective dates, the results were very similar to those obtained using only fish evacuation rates (June 28–29: 21.29%, July 26–27: 29.35%). The reasons for this consistency are likely to be the similar evacuation rates and the proportionally low amounts of shrimp consumed.

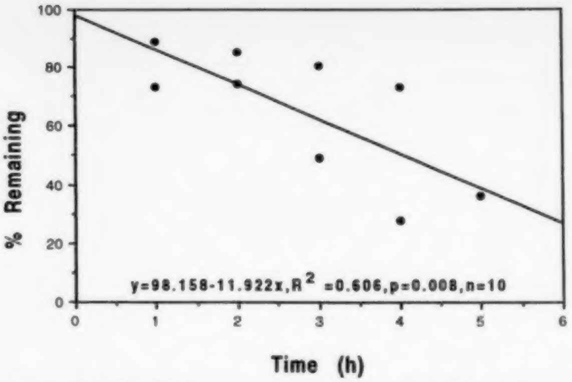

FIG. 4. Relationship between percent prey remaining and time for YOY bluefish feeding on sand shrimp. The experiment was conducted on August 3, 1990 (mean bluefish TL = 125.8 mm, range = 110–142, mean bluefish wet weight = 15.98, *n* = 10, mean prey wet weight = 0.522 g, prey weight/predator weight = 3.265%).

TABLE 1. Daily ration estimates (% body weight/d) from field-collected bluefish on two dates in 1989, using four consumption models.

| Model | June 28–29 | July 26–27 |
|---|---|---|
| Eggers | 16.78 | 24.40 |
| Elliott and Persson | 16.78 | 24.40 |
| Pennington | 32.88 | 28.81 |
| Olson and Boggs | 22.69 | 32.93 |
| Mean TL (mm) | 98.85 | 135.45 |

### Growth, Consumption, and Gross Efficiency in Laboratory Experiments

ANOVAs performed on specific growth rates calculated from wet weight and dry weight detected a significant effect of diet (wet weight: $p = 0.027$, df = 3,8, $F = 5.235$; dry weight: $p = 0.028$, df = 3,8, $F = 5.206$). The general pattern was for growth to be highest on the fish diets, intermediate on the shrimp diet, and lowest on *Artemia* (Fig. 5; Table 2). Tukey HSD multiple comparisons revealed significantly higher growth on the fish diets than on the *Artemia* diet but no significant differences in growth between shrimp and fish or among the two fish size classes. When examining absolute growth in length during the experiment, similar results were obtained, with the addition that the shrimp diet also produced significantly higher growth than the

*Artemia* diet ($p < 0.001$, df = 3,8, $F = 17.846$). However, growth was relatively high on all diets, ranging from 1.2 mm/d on *Artemia* to 2.0 mm/d on *Menidia* of medium size.

ANOVAs performed on the daily specific feeding rates (i.e., consumption) indicated a significant treatment effect (wet weight: $p < 0.001$, df = 3,8, $F = 29.266$; dry weights: $p = 0.007$, df = 3,8, $F = 8.446$; energy: $p = 0.001$, df = 3,8, $F = 14.477$). Highest feeding rates were on *Artemia*, followed by small Atlantic silverside, medium Atlantic silverside, and shrimp (Fig. 5; Table 2). Multiple comparison tests performed on dry weight consumption indicated highest feeding rates on small Atlantic silverside and *Artemia* diets, intermediate rates on medium *Menidia*, and lowest on shrimp (significantly different from both *Artemia* ($p = 0.011$) and small Atlantic silverside feeding rates ($p = 0.015$).

Gross growth efficiencies were also dependent on diet (wet weight: $p < 0.001$, df = 3,8, $F = 85.063$; dry weight: $p < 0.001$, df = 3,8, $F = 40.085$; energy: $p < 0.001$, df = 3,8, $F = 59.888$). Comparisons of means (based on dry weight) showed highest efficiencies on shrimp and medium *Menidia*, lower efficiencies on small *Menidia* (significantly different from all other diets), and lowest efficiencies on *Artemia* (significantly different from all other diets) (Fig. 5; Table 2).

The mean number of daily prey consumed per individual also depended on diet ($p < 0.001$, df = 3,8, $F = 317.778$). An individual bluefish consumed an average of 142.0 ± 7.7 (SE) *Artemia*, 4.9 ± 0.3 shrimp, 7.7 ± 0.6 small Atlantic silversides, or 2.6 ± 0.3 medium Atlantic silversides per day. Comparison of these means revealed that only the number of *Artemia* consumed was significantly different from the numbers of other prey ingested ($p < 0.0001$). If *Artemia* consumption was excluded, the ANOVA was still significant ($p < 0.001$, df = 2,6, $F = 42.258$) and all means were significantly different from each other (all $p < 0.016$).

## Discussion

### Diel Diet and Gut Fullness

YOY bluefish diets generally reflect prey relative abundances and prey-size availability (Juanes 1992; Juanes et al. 1993). In this study, the diet was predominantly piscivorous and was dominated by Atlantic silverside in the first sample and by bay anchovy and other fishes in the second sample (Fig. 1).

Feeding by YOY bluefish was continuous, but tended to be lowest just after sunrise and peaked in late afternoon. On July 26–27, a second peak was observed at 02:00. However, this sample contained significantly larger fish than those collected during daylight hours (Fig. 2). In addition, because the moon rose earlier on this second sampling date, there may have been sufficient light for visual foraging. Although bluefish are considered visual predators (Marks and Conover 1993), with activity peaking during daylight hours (Olla and Studholme 1972, 1978), laboratory experiments have shown that they are able to feed in complete darkness (F. Juanes, unpublished data).

### Evacuation Rates and Field Consumption Estimates

YOY bluefish evacuate food from their stomachs faster than most other piscivorous fishes studied (Magnuson 1969; Windell 1978; Fange and Grove 1979). Only yellowfin (*Thunnus thynnus*) and skipjack tuna (*Katsuwonus pelamis*) (Magnuson 1969; Olson and Boggs 1986) and juvenile piscivorous coho salmon (*Oncorhynchus kisutch*) (Ruggerone 1989) have been shown to have comparable evacuation times (ranging from 10 to 12 h). Similarly, the daily consumption of YOY bluefish on fish diets appears to be larger than that estimated for other piscivorous fishes (usually <5% body weight/d). The only comparable rates are for juvenile sablefish (*Anoplopoma fimbria*), an open-water epipelagic species that consumes about 30% wet body weight/d (Shenker and Olla 1986), juvenile blue marlin (*Makaira nigricans*) (30–45%, Gorbunova and Lippskaya 1975), and skipjack tuna (28–35%, Kitchell et al. 1978). Bluefish consumption estimates lie in the middle of the range outlined for tropical

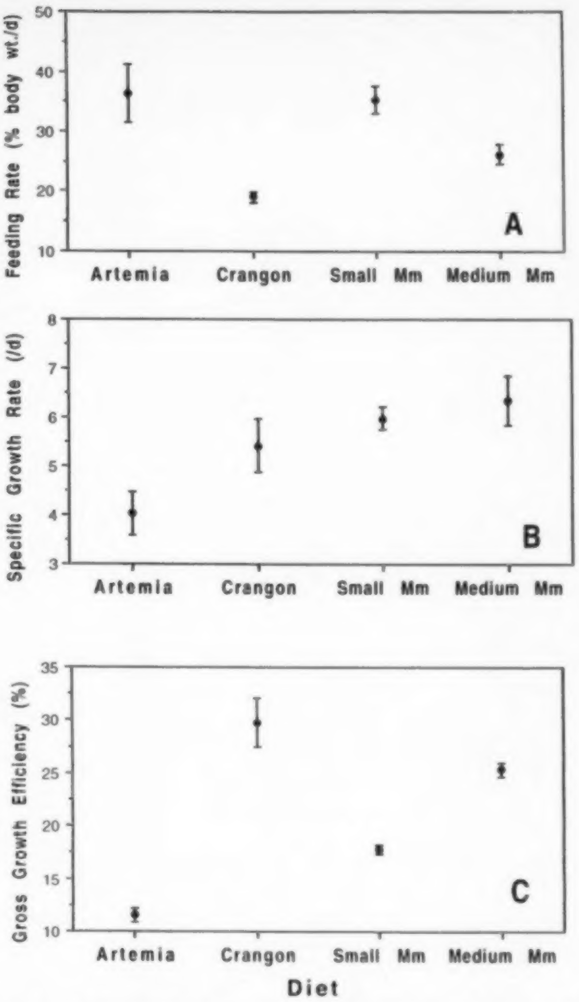

FIG. 5. Mean (A) feeding rate, (B) specific growth rate, and (C) gross growth efficiency of YOY bluefish fed on four live diets for 18 d. Each point represents the mean of three replicates. Calculations were based on dry weights. Each replicate was composed of three bluefish. Error bars are standard errors. Diets were *Artemia* adults, sand shrimp (*Crangon septemspinosa*), small *M. menidia* (*Mm*) (20–40 mm TL), and medium *Mm* (50–70 mm TL).

TABLE 2. Results of laboratory growth experiments. *F*, feeding rate (%body weight or energy content/d); *G*, specific growth rate (/d); *E*, gross growth efficiency (%); *n*, number of individuals consumed per day; BTL, bluefish size (mm) at the end of the experiment. *Mm*, *Menidia menidia*. WWT, DWT, kJ, and TL refer to measurements made using wet weights, dry weights, kilojoules, and total lengths (absolute values), respectively. See text for measurement formulas.

| | | Diet | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Artemia* | | *Crangon* | | Small *Mm* | | Medium *Mm* | |
| | Method | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| *F* | WWT | 122.78 | 18.11 | 20.40 | 0.81 | 29.98 | 1.71 | 22.61 | 1.37 |
| | DWT | 36.32 | 4.82 | 19.03 | 0.84 | 35.34 | 2.22 | 26.30 | 1.66 |
| | kJ | 14.25 | 1.89 | 4.94 | 0.22 | 12.01 | 0.75 | 8.94 | 0.56 |
| *G* | WWT | 3.87 | 0.42 | 5.21 | 0.53 | 5.75 | 0.23 | 6.10 | 0.48 |
| | DWT | 4.03 | 0.44 | 5.42 | 0.55 | 5.98 | 0.24 | 6.34 | 0.49 |
| | TL | 21.56 | 0.99 | 30.33 | 1.07 | 32.22 | 1.64 | 36.00 | 1.90 |
| *E* | WWT | 3.18 | 0.19 | 26.69 | 2.26 | 20.05 | 0.46 | 28.28 | 0.92 |
| | DWT | 11.55 | 0.69 | 29.81 | 2.31 | 17.88 | 0.45 | 25.34 | 0.71 |
| | kJ | 9.80 | 0.58 | 38.27 | 2.96 | 17.54 | 0.44 | 24.85 | 0.69 |
| *n* | | 142.01 | 7.66 | 4.88 | 0.26 | 7.70 | 0.56 | 2.64 | 0.27 |
| BTL | | 101.55 | 4.07 | 117.33 | 2.80 | 123.00 | 3.33 | 126.44 | 7.85 |

fishes (Pandian and Vivekanandan 1985). The combination of relatively high evacuation and feeding rates might be expected, since interspecific comparisons reveal an exponential relationship between gastric evacuation rate and feeding rate (Pandian and Vivekanandan 1985).

The evacuation rates and consumption rates calculated here are likely to be underestimates, since the experiments were conducted at lower temperatures than those regularly measured in GSB and temperature is known to consistently increase evacuation rates and thus daily ration estimates (Windell 1978; Persson 1982). However, evacuation rates obtained in the laboratory can also be affected by other factors such as stress and enforced starvation periods (Swenson and Smith 1973; Windell 1978; Talbot 1985).

Linear, exponential, and square root forms of the evacuation model provided similar fits to the data collected in this study. These preliminary results also indicate little effect of predator size or prey type on gastric evacuation rates. More rigorous experiments using shorter intervals (15 or 30 min), more replicates, and varying meal sizes might resolve the best form of the evacuation curve with which to describe warmwater, continuously feeding predators such as bluefish. The rapid evacuation times exhibited by bluefish precluded the use of Diana's (1979) cold-water piscivore model because it assumes digestion periods of days. In addition, because bluefish feed fairly continuously during the day and the original sizes of prey could not always be reconstructed, the Adams et al. (1982) model for warmwater piscivores could not be applied.

## Consumption Comparisons

The consumption estimates from field-collected bluefish are similar irrespective of the model used (Table 1). The only exception is the estimate from the Pennington model for the smaller fish (June 28–29 sample). A similar result was obtained by Ruggerone (1989) when comparing daily ration estimates based on linear, exponential, and square root evacuation models for juvenile coho salmon fed sockeye salmon (*Oncorhynchus nerka*) fry. He found that the Eggers and the Elliott and Persson models resulted in very similar estimates of food consumption whereas the Pennington model tended to estimate slightly lower daily rations. Boisclair and Leggett (1988), after a series of ten 24-h samplings of yellow perch (*Perca flavescens*), found that the daily ration estimates derived from both the Eggers and the Elliott and Persson models did not differ significantly. This consistency occurred despite the lack of continuous feeding or equal gut fullnesses at the beginning and end of a 24-h sampling period. The authors concluded that the Eggers model is robust and can be applied to species that feed throughout the day on a range of food types, exhibit feeding peaks, and do not have rigid feeding periodicities. Hayward (1991) has recently shown that the estimates derived from these models (also on yellow perch) tend to vary as the sampling intervals become longer than 6 h. Although the various consumption models provided similar estimates, the results of this study cannot assess the appropriateness of a particular model, since various assumptions (such as continuous feeding, full 24-h samples, equal amounts of food in the gut at the beginning and end of the cycle) may have been violated.

Field estimates were generally lower than estimates derived from laboratory experiments (for the fish diets), although it is difficult to compare the results directly, since bluefish sizes differed (Tables 1 and 2). However, laboratory bluefish sizes overlapped with those from both field samples. Sizes at the beginning of the experiment were similar to those measured on June 28–29 whereas sizes at the end of the experiment resembled those of the second diel sampling (the experiment was conducted during the first 3 wk of July 1990). Moreover, results of the laboratory evacuation experiments showed no differences between bluefish sizes. Field values are likely to be underestimated because the temperatures during both diel samplings were higher than the average temperature during either the growth experiment or the evacuation trials. Other studies have also found field estimates to be lower than laboratory daily consumption estimates (Mann 1978; Worobec 1984; Canino et al. 1991).

### Growth

Results of the laboratory growth experiments showed a significant effect of food type. Growth was highest on the fish diets, intermediate (although not significantly different from the fish diets) on shrimp, and lowest on *Artemia* (Fig. 5; Table 2). Thus, as predicted, growth on a fish diet was significantly higher than growth on a zooplanktivorous diet. Potential reasons for this growth rate difference are the increased foraging costs of feeding on *Artemia* (see below) and the higher dry to wet weight ratio of fish (0.259) and shrimp (0.231) prey as compared with *Artemia* (0.065) (Shenker and Olla 1986). Similar results were obtained by Martin (1966) when examining growth of planktivorous and piscivorous lake trout (*Salvelinus namaycush*). Those fish fed on a fish diet attained maximum weights at least 2.5 times higher than those that remained planktivorous.

Most studies of fish consumption have found a positive relationship between growth rate and feeding rate (often described as a negatively accelerated curve, Brett and Groves 1979; Wootton 1990). Recent evidence has suggested that this may not always be true. Boisclair and Leggett (1989a, 1989b, 1989c, 1989d), in a series of papers, showed a lack of correlation between field estimates of food consumption and relative growth rates of age 2 and 3 yellow perch across 12 lake populations. They suggested that differential activity costs rather than feeding rate led to the interpopulation growth differences (Boisclair and Leggett 1989d, 1990, 1991). In our study, feeding and growth rates were highest on fish diets (Fig. 5). However, the opposite pattern was found for fish fed on *Artemia*: high feeding rates but low growth rates (despite the similar caloric values of fish and *Artemia*). This result is likely due to the extreme differences between the average number of prey constituting the daily ration of each diet. Hence, *Artemia*-fed fish would incur increased foraging costs due to the increased number of feeding bouts resulting in lower growth (Mann 1978).

Despite the observed differences in growth, all the estimated growth rates were as high (*Artemia*) or higher (shrimp and fish) than that observed in the field (McBride and Conover 1991). Generally, laboratory-derived growth rates tend to be higher than those estimated from field collections because fish in the laboratory will feed at higher rates if given food ad libitum and tend to exhibit reduced activity levels from being confined to small spaces resulting in lower energy expenditures (Kerr 1971b; Mann 1978; Brett 1979; Brett and Groves 1979; Ricker 1979; Hawkins et al. 1985; Jobling 1985; Werner 1986). The growth rates of bluefish fed on fish diets observed in this study (1.8–2.0 mm/d) also rank among the highest measured for juvenile fishes (Shenker and Olla 1986; D'Amours et al. 1990; Szedlmayer et al. 1992).

### Growth Efficiency

Gross growth efficiency is the ratio of growth to consumption. The results of this study show the highest efficiences on a shrimp diet, intermediate on fish diets, and lowest on *Artemia* (Fig. 5; Table 2). All of the observed efficiencies based on dry weight (10–30%) lie in the range of those obtained for other juvenile marine fishes (Brett and Groves 1979; Lane et al. 1979; Smith et al. 1986; Malloy and Targett 1991). The comparatively low efficiencies of fish and *Artemia* diets as compared with the shrimp diet may be a function of the well-described relationship between conversion efficiency and ration in fishes (Paloheimo and Dickie 1966; Kerr 1971a, 1971b; Brett and Groves 1979). This relationship produces a humped curve in which highest efficiencies are obtained at intermediate ration levels ("optimum rations"), while increased rations lead to reduced efficiencies (the "*K*-line"). Such a relationship suggests that bluefish were consuming near-optimal rations of shrimp but maximum rations of fish and *Artemia* resulting in lower growth efficiencies.

In conclusion, YOY bluefish show relatively high evacuation rates, feeding rates, and specific growth rates that are as high as those for pelagic warmwater fish species. These high rates, combined with more rapid growth after the shift from a zooplankton to a fish diet, explain how YOY bluefish, by pursuing a tropical life-style in a temperate environment, can achieve higher growth rates and hence much greater sizes at age 1 than any other morphologically similar species in this region (Nyman and Conover 1988; Chiarella and Conover 1990; McBride and Conover 1991). This life-style may also have other ecological implications, including potentially strong effects on prey populations, particularly if predation mortality is an important determinant of recruitment success (see Juanes et al. 1993 for an example in the Hudson River estuary).

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# Genetic Analysis of Striped Marlin (*Tetrapturus audax*) Population Structure in the Pacific Ocean

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Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) was used to determine the magnitude of intraspecific genetic differentiation among samples of striped marlin (*Tetrapturus audax*). Composite genotypes, representing information from 11 restriction endonucleases, were generated for samples of approximately 40 striped marlin each from Mexico, Ecuador, Australia, and Hawaii. Genotypic variation was relatively high in all samples: the pooled nucleon diversity was 0.82 (sample range 0.69–0.84), and the pooled nucleotide sequence diversity was 0.30% (sample range 0.20–0.32%). Values of corrected mean nucleotide sequence divergence between samples were low (0.01–0.06%, mean 0.03%). Despite low intersample divergences, significant heterogeneity was observed in the distribution of composite mtDNA genotypes among samples. Each location was characterized by the presence of unique genotypes at elevated frequencies. Samples taken from the same location 1 yr apart demonstrated temporal stability of the distribution of genotypes at that site. These results demonstrate significant spatial partitioning of genetic variation within striped marlin, a situation that sharply contrasts with similar studies of several species of tuna. Management should focus on units smaller than those currently used to conserve unique genetic variation within the species.

On a fait appel à une analyse du polymorphisme de la longueur des fragments de restriction (RFLP) de l'ADN mitochondrial (ADNmt) pour déterminer l'ampleur de la différenciation génétique intraspécifique chez des échantillons de marlin rayé (*Tetrapturus audax*). Les génotypes composites, constitués de l'information obtenue au moyen de 11 endonucléases de restriction, ont été produits pour des échantillons provenant d'une quarantaine de marlins rayés capturés dans chacun des pays suivants : Mexique, Équateur, Australie et Hawaii. La variation du génotype était relativement élevée dans tous les échantillons : la diversité de l'ensemble du nucléon était 0,82 (0,69–0,84), et la diversité de l'ensemble des séquences de nucléotides était 0,30 % (0,20–0,32 %). Les valeurs de la divergence moyenne corrigée des séquences de nucléotides entre les échantillons étaient faibles (0,01 à 0,06 %, moyenne de 0,03 %). Malgré les faibles divergences entre chaque échantillon, on a observé une hétérogénéité significative dans la distribution des génotypes composites d'ADNmt des échantillons. Chaque endroit se caractérisait par la présence de génotypes uniques à des fréquences élevées. La distribution des génotypes est restée stable chez les échantillons prélevés au même endroit à 1 an d'intervalle. Ces résultats montrent une distribution spatiale significative de la variation génétique chez le marlin rayé, une observation qui va tout à fait à l'encontre d'études similaires effectuées chez plusieurs espèces de thon. La gestion doit tenter de conserver des unités plus petites que celles utilisées jusqu'à maintenant pour conserver l'unique variation génétique au sein de l'espèce.

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The striped marlin (*Tetrapturus audax*) occurs throughout the tropical, subtropical, and temperate waters of the Pacific and Indian oceans (Nakamura 1985), with occasional catches reported from the Atlantic Ocean near the Cape of Good Hope (Talbot and Penrith 1962). In the Pacific Ocean, the highest concentration of striped marlin occurs in a horseshoe-shaped pattern (Fig. 1) with a base along the Central American coast and extensions across the North and South Pacific (Nakamura 1985).

Striped marlin are an important commercial and recreational fisheries resource throughout their range. In the eastern Pacific, they are the principal target of a longline fishery and are a frequent bycatch of longline gear deployed for tunas in other parts of their range (Nakamura 1974). The greatest catch of striped marlin occurs in the North Pacific; the South Pacific generally contributes approximately 20–25% and the Indian Ocean about 15–20% of the total (FAO 1992). Between 1980 and 1990, annual landings of striped marlin fluctuated between 12 600 and 18 300 metric tons (FAO 1992).

The stock structure and movements of striped marlin in the Pacific Ocean were recently summarized by Squire and Suzuki (1990); they reviewed tag/recapture and morphometric analyses of striped marlin, as well as studies of the

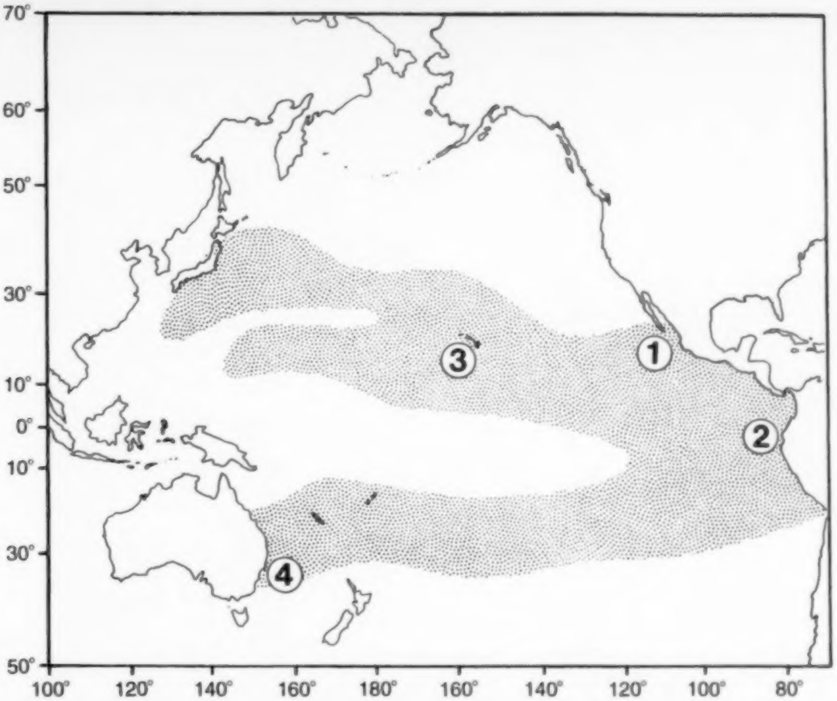

FIG. 1. *Tetrapturus audax* sampling locations: 1, Cabo San Lucas, Mexico; 2, Manta, Ecuador; 3, Kona, Hawaii; 4, Port Stephens, Australia.

spatial/temporal patterns of reproductive condition/larval abundance and catch per unit effort (CPUE). The authors concluded that there was considerable evidence of movement of individuals between geographically distant areas, and consequently a general lack of stock structure. Their findings were similar to those of Shomura (1980), who proposed dividing the Pacific striped marlin resource into a North and South Pacific stock for assessment purposes.

There are very few published accounts of the genetic basis of population structure of any billfish species and none of the striped marlin. Although large marine vertebrates were originally reported to lack substantial genetic variation (Sharp, as cited in Selander 1976), preliminary genetic analyses of billfish population structure have revealed substantial variation using a variety of techniques including allozyme analysis (Shaklee et al. 1983; Morgan 1992) and direct sequence analysis of a 406-bp region of the mitochondrial DNA (mtDNA) cytochrome *b* gene (Finnerty and Block 1992).

The selection of an appropriate genetic character and method of analysis is essential for the success of any population genetic study. Restriction fragment length polymorphism (RFLP) analysis of mtDNA has been successfully used to study the population structure of several marine fishes because it tends to reveal considerable variation (Ovenden 1990); however, the technique has not been appropriate for some studies due to insufficient or excessive restriction site variation (Kornfield and Bogdanowicz 1987; Wirgin et al. 1988; Bowen and Avise 1990; Chapman 1990). In this investigation, we used RFLP analysis of mtDNA to test the null hypothesis that striped marlin within the Pacific Ocean share a common gene pool.

## Materials and Methods

Samples of approximately 40 striped marlin each were obtained from sportfishing and artisinal fishing operations at Cabo San Lucas, Mexico (MEX); Manta, Ecuador (ECU); Kona, Hawaii (HAW); and Port Stephens, Australia (AUS) over a 2-yr period (Fig. 1; Table 1). Heart tissue was removed from specimens within 8 h of capture and placed immediately on crushed ice. Most tissue samples were subsequently frozen in a standard freezer (−10 to −20°C) within a few hours of dissection and transported from collection locations to the laboratory on dry ice or commercial ice substitutes. Tissues were maintained in the laboratory at −70°C until processing.

For most samples, mtDNA was purified using the equilibrium density gradient centrifugation protocols of Lansman et al. (1981). Aliquots of mtDNA were individually digested with the following 11 restriction endonucleases following the manufacturer's instructions: *Apa*I, *Ava*I, *Ava*II, *Ban*I, *Bcl*I, *Bgl*I, *Bst*EII, *Hae*II, *Hind*III, *Sca*I, and *Sst*II. The resultant restriction fragments were endlabeled with $^{35}$S-labeled nucleotide triphosphates using the Klenow fragment, separated electrophoretically at 2 V/cm overnight on 0.8–1.5% agarose gels, and visualized by autoradiography (Sambrook et al. 1989).

Yields of supercoiled mtDNA were low from one shipment of 20 striped marlin hearts from Kona, Hawaii, that thawed twice before arriving at the laboratory and from the sample of striped marlin from Mexico which remained on ice at the collection location for 1 wk before it could be frozen. For these samples, the upper band in the cesium chloride gradient containing both nuclear DNA and relaxed mtDNA

TABLE 1. Striped marlin collection information (additonal data (individual weight, sex, and mtDNA genotype) are available from the authors upon request).

| Location | | *N* | Date | Source |
|---|---|---|---|---|
| Cabo San Lucas, B.C.S., Mexico | MEX | 36 | June 1991 | Sport fish |
| Manta, Ecuador | ECU | 40 | Apr.–Oct. 1990 | Artisinal fishery |
| Kona, Hawaii | HAW | 43 | June–Nov. 1991 | Sport fish |
| Port Stephens, N.S.W., Australia | AUS | 47 | Feb. 1991, Feb. 1992 | Sport fish |

TABLE 2. Distribution of striped marlin mtDNA genotypes among four Pacific sampling locations. Composite genotypes reflect digestions with the following restriction endonucleases (left to right): *Ava*I, *Ban*I, *Bcl*I, *Bgl*I, *Bst*EII, *Hae*II, *Hind*III, *Sst*II, *Sca*I, *Apa*I, and *Ava*II.

| No. | Genotype | MEX | ECU | HAW | AUS | Total |
|---|---|---|---|---|---|---|
| 1 | AAAAAAAAAAA | 12 | 20 | 14 | 12 | 58 |
| 2 | AAAAAAAAAAB | 6 | 6 | 7 | 3 | 22 |
| 3 | AAAAAAAAAAC | 8 | 9 | 4 | 1 | 22 |
| 4 | AAAAAABAAAB | 0 | 2 | 10 | 8 | 20 |
| 5 | BAAAAAAAAAA | 0 | 1 | 2 | 7 | 10 |
| 6 | BAAAAAAABAA | 0 | 1 | 0 | 11 | 12 |
| 7 | ABAAAAAAAAA | 6 | 0 | 0 | 0 | 6 |
| 8 | BABAAAAAAAA | 0 | 0 | 3 | 0 | 3 |
| 9 | ACAAAABAAAB | 0 | 0 | 0 | 2 | 2 |
| 10 | CAAAACBAAAB | 0 | 0 | 1 | 0 | 1 |
| 11 | AAABAAAAAAB | 0 | 0 | 1 | 0 | 1 |
| 12 | CAAAAAAAAAB | 0 | 0 | 1 | 0 | 1 |
| 13 | AAAAACAAAAA | 1 | 0 | 0 | 0 | 1 |
| 14 | ACAAAAAAAAB | 1 | 0 | 0 | 0 | 1 |
| 15 | ABAAABAAAAB | 1 | 0 | 0 | 0 | 1 |
| 16 | BAAAAACAAAA | 0 | 0 | 0 | 1 | 1 |
| 17 | AABAAAAAAAA | 0 | 0 | 0 | 1 | 1 |
| 18 | AAABAAAAABB | 0 | 0 | 0 | 1 | 1 |
| 19 | AAAAABAAAAB | 0 | 1 | 0 | 0 | 1 |
| 20 | AAAAAABAAAA | 1 | 0 | 0 | 0 | 1 |
| | Total | 36 | 40 | 43 | 47 | 166 |

was saved and dialyzed (Lansman et al. 1981). Alternatively, genomic DNA enriched for mtDNA was isolated following a modification of the Chapman and Powers (1984) protocol. Aliquots of these samples were digested with restriction endonucleases as above and the fragments separated electrophoretically. After electrophoresis the gels were transferred to a solid support (Southern transfer) and hybridized following the protocols of Sambrook et al. (1989). Highly purified white marlin (*Tetrapturus albidus*) mtDNA, nick translated with biotin-7-dATP, was used as a probe for mtDNA fragments. Hybridization filters were visualized after stringency washes following the procedures of the BRL BluGene Non-Radioactive Nucleic Acid Detection Kit.

Each different fragment pattern produced by a restriction endonuclease was assigned a letter. A composite mtDNA genotype, consisting of 11 letters representing the fragment patterns generated by each of the restriction endonucleases, was compiled for each individual. The nucleon diversity ($h$) was calculated following Nei (1987). The nucleotide sequence divergence ($p$) among mtDNA genotypes was estimated from a restriction site presence/absence martrix using the restriction enzyme analysis package (REAP) of McElroy et al. (1992) which uses the approach of Nei and Miller (1990). Mean nucleotide sequence divergences, within and between striped marlin samples, were calculated following the method of Nei (1987), and the divergences between samples were corrected for within-sample diversities. The distribution of genotypes among collections was evaluated for homogeneity using the chi-square randomization method of Roff and Bentzen (1989), which allows for the incorporation of rare genotypes.

## Results

The 11 restriction endonucleases used to screen striped marlin mtDNA revealed a total of 20 genotypes among the 166 individuals (Table 2). One genotype was found in 58 of the 166 fish (0.35), while 11 genotypes occurred but once. Two of the 11 restriction endonucleases (*Bst*EII and *Sst*II) revealed no variation among the 166 individuals, while the remaining nine enzymes revealed two or three distinct fragment patterns that could be directly attributed to the gain or loss of restriction sites (Table 3). No size variation or heteroplasmy was noted.

Considerable genetic variation was encountered within each striped marlin sample. Nucleon diversities ranged from 0.69 (ECU) to 0.84 (AUS), with a value for the pooled samples of 0.82 (Table 4). Although several genotypes were encountered within each sample, most genotypes differed by less than three restriction site changes, so the sequence divergence between various genotypes was generally not very large (Table 5). The nucleotide sequence divergences between the 20 genotypes ranged from 0.16 to 1.11%, with a mean of 0.53 (Table 5). As a consequence of the low degree of divergence between genotypes, within-sample mean nucleotide sequence diversities were not large, ranging from 0.20% (ECU) to 0.32% (AUS), with a value for the pooled samples of 0.30% (Table 4).

The mean nucleotide sequence divergences between samples were not appreciably larger than within-sample diversities (Table 6), ranging from 0.24 to 0.37%. When corrected for within-sample diversities (Nei 1987) the divergences ranged from 0.01 to 0.06% (Table 6).

Although nucleotide sequence divergences among samples were not large, spatial partitioning of the different mtDNA genotypes was evident among the four sampling locations. The frequency of the most common genotype (genotype 1, Table 2) varied considerably among locations, ranging in frequency from 0.26 (AUS) to 0.50 (ECU). Genotype 4, which differed from the common genotype by the gain of a *Hind*III site, was only represented in two individuals from the eastern Pacific (0.03), while it occurred in 18 individuals in the combined HAW and AUS samples (0.20). Conversely, genotype 3, which differed from the common genotype by a site change for *Ban*I, was present in 17 of 76 eastern Pacific fish (0.22), but was encountered in only five of the 90 central and western Pacific individuals (0.06).

While the presence of some genotypes appeared to characterize samples from the eastern and combined central and western Pacific, other genotypes were primarily restricted to single samples. Genotype 6, which differed from the common pattern by a site gain for *Ava*I and a site loss for *Sca*I, was found in a total of 11 individuals, 10 of which occurred in the AUS sample. Similarly, genotype 7 occurred in six individuals, all from the MEX sample.

The apparent spatial partitioning of genotypes among the samples was confirmed by an analysis of heterogeneity. The chi-square value calculated for the observed distribution of genotypes from all locations was considerably greater than any of the values produced during the 1000 randomizations of the data set ($p < 0.001$). Of the six possible pairwise comparisons between sampling locations, four produced chi-square values greater than any derived from the 1000 randomizations per comparison. Analysis of heterogeneity for MEX/ECU and HAW/ECU produced observed chi-square values that were exceeded in only 23 and 25 of the 1000 randomizations, respectively. Thus the null hypothesis, that striped marlin in the Pacific Ocean share a common gene pool, was rejected.

To evaluate the temporal stability of the genotypic differences among the collection locations, replicate samples of striped marlin hearts were collected from Port Stephens, N.S.W., Australia, in 1991 and 1992. Due to differences in the availability of striped marlin between the two years, samples were of different sizes (1991, $N = 9$; 1992, $N = 38$). Genotypes 5 and 6, which were present at low frequencies outside the AUS samples, occurred in two and four individuals, respectively, in the small 1991 AUS sample. These same two genotypes were present in five and seven individuals, respectively, in the larger 1992 sample. Thus, genotypes characteristic of the 1991 sample were also present in the 1992 sample. Chi-square heterogeneity analysis of the genotypic distribution of the two samples using the Roff and Bentzen (1989) protocol produced chi-square values greater than the observed value 805 out of 1000 times. As a result, the two samples were pooled for geographic analyses.

TABLE 3. Digestion patterns of striped marlin produced by 11 restriction endonucleases. Fragment sizes are in kilobase pairs.

| *Ava*I | | | *Ban*I | | |
|---|---|---|---|---|---|
| A | B | C | A | B | C |
| 10.30 | 10.30 | 10.30 | 7.32 | 7.32 | 7.32 |
| 3.37 | 3.37 | 3.13 | 3.45 | 5.67 | 5.67 |
| 3.13 | 2.41 | 2.57 | 2.22 | 1.72 | 1.72 |
| | 0.72 | 0.80 | 1.72 | 1.59 | 1.26 |
| | | | 1.26 | 0.50 | 0.50 |
| | | | 0.50 | | 0.33 |
| | | | 0.33 | | |

| *Bcl*I | | *Bgl*I | | *Bst*EII |
|---|---|---|---|---|
| A | B | A | B | A |
| 10.79 | 6.47 | 7.60 | 10.46 | 7.44 |
| 5.05 | 5.05 | 4.64 | 4.64 | 6.72 |
| 0.76 | 4.32 | 2.86 | 0.80 | 1.56 |
| | 0.76 | 0.80 | | 0.99 |

| *Hae*II | | | *Hind*III | | | *Sst*II |
|---|---|---|---|---|---|---|
| A | B | C | A | B | C | A |
| 6.44 | 6.44 | 6.44 | 8.66 | 6.88 | 8.66 | 14.90 |
| 3.47 | 5.04 | 3.49 | 6.88 | 6.88 | 5.38 | 1.83 |
| 2.86 | 3.47 | 3.47 | 0.80 | 1.78 | 1.50 | |
| 2.18 | 1.31 | 2.86 | 0.30 | 0.80 | 0.80 | |
| 1.31 | 0.50 | 0.50 | | 0.30 | 0.30 | |
| 0.50 | | | | | | |

| *Sca*I | | *Apa*I | | *Ava*II | | |
|---|---|---|---|---|---|---|
| A | B | A | B | A | B | C |
| 4.28 | 5.70 | 8.20 | 8.20 | 3.32 | 5.15 | 5.15 |
| 3.86 | 3.86 | 3.65 | 3.91 | 2.53 | 2.53 | 2.53 |
| 2.87 | 2.87 | 2.84 | 3.65 | 2.07 | 2.07 | 2.07 |
| 1.64 | 1.64 | 1.07 | 0.63 | 1.93 | 1.93 | 1.93 |
| 1.42 | 1.35 | 0.63 | 0.40 | 1.83 | 1.33 | 1.73 |
| 1.35 | 0.80 | 0.40 | | 1.33 | 1.00 | 1.00 |
| 0.80 | 0.46 | | | 1.00 | 0.40 | |
| 0.46 | | | | 0.40 | | |

## Discussion

Relative to other marine fishes, striped marlin exhibit substantial mtDNA variation. Comparison of nucleon diversities among studies is difficult because the value is quite sensitive to the number of restriction sites surveyed (number of enzymes used) and is biased upward if only variant enzymes are included in the analysis. With the above constraints in mind, the nucleon diversity of striped marlin (0.82) fell within the upper part of the range (0.473–0.998) reported by Avise et al. (1989) for other fishes. Likewise, the mean nucleotide sequence diversity, which can be more easily compared with other studies (although still sensitive to the preselection of variant enzymes), was in the midrange of values reported for other species of marine fishes (Ovenden 1990).

TABLE 4. Comparison of measures of mtDNA diversity and divergence within and between samples of striped marlin and yellowfin tuna collected from the same four locations in the Pacific Ocean. Yellowfin tuna data are from Scoles and Graves (1993).

|  | *N* | Nucleon diversity | Mean nucleotide sequence diversity (%) | Corrected mean nucleotide sequence divergence (%) |
|---|---|---|---|---|
| Striped marlin | 166 | 0.82 | 0.30 | 0.03 |
| Mexico | 36 | 0.80 | 0.30 | — |
| Ecuador | 40 | 0.69 | 0.20 | — |
| Hawaii | 43 | 0.80 | 0.27 | — |
| Australia | 47 | 0.84 | 0.32 | — |
| Yellowfin tuna | 80 | 0.84 | 0.28 | 0.00 |
| Mexico | 20 | 0.86 | 0.39 | — |
| Ecuador | 20 | 0.86 | 0.37 | — |
| Hawaii | 20 | 0.87 | 0.32 | — |
| Australia | 20 | 0.81 | 0.37 | — |

A surprising degree of spatial partitioning of the observed genetic variation was noted among the sampling sites. Each sample was characterized by the presence of different genotypes at relatively high frequencies. Due to the low mean nucleotide sequence divergence among these genotypes (typically one or two restriction site changes) and the high level of within-sample variation, the corrected mean nucleotide sequence divergences between samples were not large. Nevertheless, the distribution of mtDNA genotypes among collections was highly heterogeneous.

Analysis of samples from Australia collected over two consecutive years indicated that there is temporal stability of genotypic distributions at a single collection site. Those genotypes that characterized the small sample of Australian striped marlin in 1991 were also present in the larger 1992 sample. Although no significant difference in the distribution of genotypes between the temporal samples was detected, the power of the test was limited by the small sample size of the 1991 collection. Effective examination of the temporal stability of the genotypic distributions will require larger sample sizes over a much longer period.

Population structure analyses of other pelagic marine fishes have shown substantial genetic variation, but there has generally been little, if any, spatial partitioning of the variation. Allozyme analyses of pelagic species such as skipjack tuna (*Katsuwonus pelamis*) (Fujio et al. 1981) and dolphin (*Coryphaena hippurus*) (Rosenblatt and Waples 1986) have revealed minimal genetic differentiation between conspecific samples from the Atlantic and Pacific oceans. Similarly, studies employing RFLP analysis of mtDNA to compare small samples of Atlantic and Pacific skipjack tuna (Graves et al. 1984), albacore (*Thunnus alalunga*) (Graves and Dizon 1989), and dolphin (C. Reeb, University of Hawaii, personal communication) could not differentiate fish from different oceans. In contrast, there was significant heterogeneity among the geographically distant samples of striped marlin from the Pacific Ocean.

The large difference in population structure between striped marlin and some tunas is best demonstrated by comparing our results with those of a recent analysis of yellowfin tuna (*Thunnus albacares*) population structure within the Pacific Ocean (Scoles and Graves 1993). Samples of 20 yellowfin tuna each were collected from the same four locations as the striped marlin and analyzed with a suite of 12 restriction enzymes. Levels of overall genetic diversity were similar for striped marlin and yellowfin tuna (Table 4), as were the corrected mean nucleotide sequence divergences between samples, but the distribution of genotypes among the four collection sites was quite different for the two species (Table 4). Among the yellowfin tuna samples, no heterogeneity was observed in the distribution of genotypes ($p = 0.52$), while the striped marlin genotypes displayed significant heterogeneity ($p < 0.001$).

The large difference in levels of population structure between striped marlin and yellowfin tuna within the Pacific Ocean is somewhat surprising. Both species have pelagic larvae and are known to undertake long-distance movements, characteristics that would tend to promote gene flow and reduce genetic heterogeneity, the apparent condition for yellowfin tuna. What, then, promotes genetic differentiation among striped marlin?

One mechanism that could promote genetic differentiation among striped marlin is spawning site fidelity. Tag and recapture studies have demonstrated that some striped marlin undertake long-distance movements; however, a large fraction of fish are recovered in the same area in which they were tagged, even after several years at liberty (Squire and Suzuki 1990). While the nature of the movements is not well understood, owing in part to a patchy distribution of tagging effort and a low rate of tag recoveries, site fidelity cannot be dismissed. Whether this occurs for spawning sites is not known, but an annual migration of striped marlin from the Cabo San Lucas area to the southwest, an area of striped marlin spawning, has been reported from tag and recapture analyses (Squire and Suzuki 1990).

Striped marlin are not alone among the Istiophoridae in displaying spatial genetic heterogeneity. Finnerty and Block (1992) recently reported significant differences between Atlantic and Pacific blue marlin (*Makaira nigricans*) in the distribution of mtDNA haplotypes generated by sequence analysis of a 612-bp region of the cytochrome *b* gene. In addition, Graves and McDowell (1994) found significant

TABLE 5. Genetic divergence between striped marlin composite genotypes. Mean nucleotide sequence divergences ((%) are presented above the diagonal and minimum numbers of restriction site changes are presented below the diagonal.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | — | 0.17 | 0.36 | 0.34 | 0.17 | 0.33 | 0.34 | 0.32 | 0.51 | 0.68 | 0.34 | 0.34 | 0.17 | 0.34 | 0.72 | 0.33 | 0.16 | 0.52 | 0.34 | 0.16 |
| 2 | 1 | — | 0.18 | 0.17 | 0.34 | 0.51 | 0.52 | 0.50 | 0.34 | 0.51 | 0.17 | 0.17 | 0.34 | 0.17 | 0.55 | 0.50 | 0.34 | 0.35 | 0.17 | 0.34 |
| 3 | 2 | 1 | — | 0.34 | 0.53 | 0.70 | 0.72 | 0.69 | 0.52 | 0.69 | 0.35 | 0.34 | 0.54 | 0.35 | 0.73 | 0.69 | 0.52 | 0.53 | 0.35 | 0.52 |
| 4 | 2 | 1 | 2 | — | 0.50 | 0.67 | 0.69 | 0.66 | 0.17 | 0.34 | 0.34 | 0.33 | 0.51 | 0.34 | 0.71 | 0.66 | 0.50 | 0.52 | 0.34 | 0.17 |
| 5 | 1 | 2 | 3 | 3 | — | 0.16 | 0.53 | 0.16 | 0.68 | 0.85 | 0.51 | 0.51 | 0.34 | 0.52 | 0.90 | 0.16 | 0.33 | 0.69 | 0.52 | 0.33 |
| 6 | 2 | 3 | 4 | 4 | 1 | — | 0.70 | 0.33 | 0.85 | 1.02 | 0.69 | 0.68 | 0.51 | 0.69 | 1.08 | 0.33 | 0.50 | 0.87 | 0.69 | 0.50 |
| 7 | 2 | 3 | 4 | 4 | 3 | 3 | — | 0.69 | 0.52 | 1.06 | 0.70 | 0.70 | 0.54 | 0.35 | 0.36 | 0.69 | 0.51 | 0.89 | 0.72 | 0.51 |
| 8 | 2 | 3 | 4 | 4 | 1 | 2 | 4 | — | 0.84 | 1.00 | 0.67 | 0.66 | 0.50 | 0.68 | 1.06 | 0.32 | 0.16 | 0.85 | 0.68 | 0.49 |
| 9 | 3 | 2 | 3 | 1 | 4 | 5 | 3 | 5 | — | 0.52 | 0.52 | 0.51 | 0.69 | 0.17 | 0.53 | 0.84 | 0.67 | 0.70 | 0.52 | 0.34 |
| 10 | 3 | 3 | 4 | 2 | 5 | 6 | 6 | 6 | 3 | — | 0.69 | 0.33 | 0.51 | 0.70 | 1.11 | 1.00 | 0.84 | 0.87 | 0.70 | 0.51 |
| 11 | 2 | 1 | 2 | 2 | 3 | 4 | 4 | 4 | 3 | 4 | — | 0.34 | 0.52 | 0.35 | 0.73 | 0.67 | 0.51 | 0.17 | 0.35 | 0.51 |
| 12 | 2 | 1 | 2 | 2 | 3 | 4 | 4 | 4 | 3 | 2 | 2 | — | 0.52 | 0.35 | 0.74 | 0.66 | 0.50 | 0.52 | 0.35 | 0.50 |
| 13 | 1 | 2 | 3 | 3 | 2 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | — | 0.53 | 0.93 | 0.50 | 0.33 | 0.70 | 0.53 | 0.33 |
| 14 | 2 | 1 | 2 | 2 | 3 | 4 | 2 | 4 | 1 | 4 | 2 | 2 | 3 | — | 0.36 | 0.68 | 0.51 | 0.53 | 0.35 | 0.51 |
| 15 | 4 | 3 | 4 | 4 | 5 | 6 | 2 | 6 | 3 | 6 | 4 | 4 | 5 | 2 | — | 1.06 | 0.88 | 0.92 | 0.36 | 0.88 |
| 16 | 2 | 3 | 4 | 4 | 1 | 2 | 4 | 2 | 5 | 6 | 4 | 4 | 3 | 4 | 6 | — | 0.49 | 0.85 | 0.68 | 0.49 |
| 17 | 1 | 2 | 3 | 3 | 2 | 3 | 3 | 1 | 4 | 5 | 3 | 3 | 2 | 3 | 5 | 3 | — | 0.69 | 0.51 | 0.33 |
| 18 | 3 | 2 | 3 | 3 | 4 | 5 | 5 | 5 | 4 | 5 | 1 | 3 | 4 | 3 | 5 | 5 | 4 | — | 0.53 | 0.69 |
| 19 | 2 | 2 | 2 | 2 | 3 | 4 | 4 | 4 | 3 | 4 | 2 | 2 | 3 | 2 | 2 | 4 | 3 | 3 | — | 0.51 |
| 20 | 1 | 2 | 3 | 1 | 2 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 2 | 3 | 5 | 3 | 2 | 4 | 3 | — |

TABLE 6. Percent mean nucleotide sequence divergences between striped marlin collections. Values below the diagonal are corrected for within-sample diversities.

| | MEX | ECU | HAW | AUS |
|---|---|---|---|---|
| MEX | — | 0.26 | 0.31 | 0.37 |
| ECU | 0.01 | — | 0.25 | 0.30 |
| HAW | 0.03 | 0.01 | — | 0.32 |
| AUS | 0.06 | 0.04 | 0.03 | — |

differentiation between Atlantic and Pacific samples of blue marlin and sailfish (*Istiophorus platypterus*) using RFLP analysis of mtDNA.

Pelagic organisms have traditionally been viewed as having very low levels of population structure, resulting from the high dispersal abilities of various life history stages and the lack of physical barriers in the environment. The striped marlin, in contrast with many previously studied pelagic marine fishes, displays considerable genetic heterogeneity or stock structure. As management of this resource is currently based on the assumption of basin-wide stocks, the potential exists for the loss of unique genetic variation due to overexploitation within an area. To conserve genetic variation, it is essential that international management of striped marlin focus on smaller, genetically homogeneous stocks.

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# *Myriophyllum spicatum* Pumps Essential and Nonessential Trace Elements from Sediments to Epiphytes[1]

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To test the hypothesis that elements can be transferred from freshwater sediments to macrophytes and to epiphytes, we grew *Myriophyllum spicatum* in natural sediments containing $^{60}$Co and $^{54}$Mn (essential) and $^{137}$Cs, $^{152}$Eu, $^{154}$Eu, and $^{155}$Eu (nonessential). We found no differences between shoot or epiphyte to sediment bioaccumulation factors (BAFs) and only found differences between root and shoot to sediment BAFs for $^{60}$Co and $^{54}$Mn, probably because these elements adsorb onto root surfaces. We found no differences in BAFs for any elements between shoots and epiphytes, suggesting no differences in the transfer of essential and nonessential elements. There were, however, interelement differences for the roots, again probably due to surface adsorption related to redox sensitivity. Using isotope specific activity ratios, we calculated that over 75% of the Co and 100% of the Mn in *M. spicatum* was obtained from the sediments and that all Co and Mn in the epiphytes was sediment derived through the host. Although the net flux of trace elements from the sediments to the water via macrophytes has been shown to be small in most situations, buried pulses of nonessential elements may be reintroduced to the littoral food web by epiphytes being grazed by invertebrates and fish.

Pour vérifier l'hypothèse que les éléments peuvent passer des sédiments d'eau douce aux macrophytes et aux épiphytes, nous avons cultivé *Myriophyllum spicatum* dans des sédiments naturels renfermant $^{60}$Co et $^{54}$Mn (essentiels) ainsi que $^{137}$Cs, $^{152}$Eu, $^{154}$Eu et $^{155}$Eu (non essentiels). Nous n'avons trouvé aucune différence entre le facteur de bioaccumulation (FBA) observé dans les pousses ou les épiphytes par rapport aux sédiments, sauf dans le cas des FBA du $^{60}$Co et du $^{54}$Mn dans les racines et les pousses par rapport aux sédiments, probablement parce que ces éléments s'adsorbent à la surface des racines. Nous n'avons trouvé aucune différence de FBA pour aucun élément entre les racines et les épiphytes, ce qui indique qu'il n'y a aucune différence de transfert entre les éléments essentiels et les éléments non essentiels. Il y avait toutefois des différences entre les éléments dans les racines, ce qui encore une fois est probablement dû à l'adsorption en surface liée à la sensibilité au potentiel d'oxydo-réduction. En utilisant les rapports d'activités spécifiques des isotopes, nous avons calculé que plus de 75% du Co et que 100% du Mn mesurés dans *M. spicatum* provenaient des sédiments et que le Co et le Mn dans les épiphytes provenaient des sédiments par l'entremise de la plante hôte. Bien qu'on ait montré que le flux net des éléments en traces des sédiments vers l'eau par l'entremise des macrophytes est faible dans la plupart des cas, certaines quantités d'éléments non essentiels présents dans les sédiments peuvent être réintroduites dans la chaîne alimentaire du littoral grâce aux épiphytes dont se nourrissent les invertébrés et les poissons.

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It is well known that rooted submerged macrophytes can derive much of their nutrition from the sediments within which they are rooted (Carignan and Kalff 1979, 1980; Barko and Smart 1981; Jackson et al. 1991, 1993). However, there is no direct evidence that macrophytes derive elements not essential for their growth from their substratum. If macrophytes do take up nonessential elements directly from freshwater sediments, then such sediments represent a large potential source of elements, and possibly contaminants, available to littoral food webs because macrophytes are grazed directly by, e.g., waterflowl (Fassett 1957) and invertebrates (Lodge 1988).

Fates for elements within macrophyte tissues, in addition to direct grazing, include return to littoral sediments bound to senescing shoot fragments and loss to the water column via exudation (Jackson et al. 1994). Some essential macronutrients, e.g., C, N, and P, have been shown experimentally to

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TABLE 1. Average radionuclide concentrations in the Ottawa River sediments used for this experiment ($n = 6$). No activity levels of these elements were detected in the control sediments.

| Radioisotope | Activity (Bq/g) | SD |
|---|---|---|
| $^{60}$Co | 22.69 | 3.94 |
| $^{137}$Cs | 31.26 | 3.41 |
| $^{152}$Eu | 4.84 | 0.57 |
| $^{154}$Eu | 1.94 | 0.38 |
| $^{155}$Eu | 0.40 | 0.01 |
| $^{54}$Mn | 0.73 | 0.27 |

be taken up by epiphytes following release from the host macrophyte (Allen 1971; Harlin 1973; Carignan and Kalff 1982; Moeller et al. 1988; Burkholder and Wetzel 1989, 1990), although under some conditions the macrophytes are effectively a neutral substrate (Cattaneo and Kalff 1979). If epiphytes also intercept nonessential elements that are exuded or leak from the host macrophyte, then the macrophytes would function as a vector between sediment-bound nonessential element pools (and contaminants) and their epiphytes.

The purpose of this study was to test the hypotheses that (i) macrophytes obtain trace elements directly by root uptake from the sediments and (ii) epiphytes receive trace elements from their host macrophyte. We tested these hypotheses by growing *Myriophyllum spicatum* side by side in natural lake sediments and experimental sediments containing trace element radionuclides. We hypothesized that if direct uptake of elements from the sediments by roots followed by translocation to shoots is the main source of elements to the macrophytes (versus shoot uptake of elements from the water following release from the sediments), then only the experimental plants should contain radionuclides. We also hypothesized that if macrophytes were unable to differentiate between essential and nonessential elements, then there should be no differences in the shoot/sediment bioaccumulation factors (BAFs). In addition, we hypothesized that if elements released from macrophytes were the main source of elements to epiphytes, then epiphyte/sediment BAFs should not differ from shoot/sediment BAFs. Finally, if epiphytes were unable to prevent the uptake of nonessential elements, there should be no between-element differences in epiphyte/sediment BAFs.

## Materials and Methods

### Study Site and Sampling Protocol

This study was performed in McPherson Bay (45°06′N, 76°16′W) in the mesotrophic central basin (Chl *a* approximately 10–15 μg/L) of Lake Memphremagog (Quebec–Vermont). In early June 1991, divers collected the tips (approximately 6 cm) of sprouting *M. spicatum* from an existing weedbed and allowed them to float in a plastic wading pool until roots developed (approximately 10–14 d). The small plants ($n = 45$) were then planted in 10-cm-diameter plastic pots ($n = 9$) containing sediments that had been collected from the Ottawa River, Canada, and contained low levels (Table 1) of $^{60}$Co, $^{54}$Mn, $^{137}$Cs, $^{152}$Eu, $^{154}$Eu, and $^{155}$Eu discharged from a nuclear reactor over a period of 40 yr. Pots were then placed in small excavated holes in the naturally occurring weedbed on 16 June and three pots were removed after 32 (17 July), 66 (14 August), and 98 d (18 September).

Existing *M. spicatum* growing in natural, unlabelled lake sediments were used as controls. The closest (25 cm maximum distance away) five plants (and their epiphytes) to the experimental pots were sampled on the same dates as the experimental plants and epiphytes. The majority of control plants were typically 1–10 cm away from the pots containing the labelled sediments; we assume that this close physical proximity of control and experimental plants to the labelled sediments resulted in relatively equivalent exposure of experimental and control shoots and epiphytes to any radionuclide that might have diffused out of the labelled sediments into the water column.

In the field, divers collected the experimental (in pots) and control (in lake sediments) *M. spicatum* and associated epiphytes (primarily pennate diatoms but a few colonies of *Gleotrichia pisum* on 17 July and colonies of *G. pisum* and *Coleochaete* spp. on 14 August and 18 September) by cutting the macrophyte shoots at the base of the plant and gently placing the shoots (and intact epiphytes) in large (27 × 28 cm) heavy-duty zip-lock freezer bags. A small quantity of the loose epiphyte material was lost during this procedure, but appeared to be insignificant compared with the amount of material retained on the shoots. Roots were then removed from the experimental (in pots) and control (in lake) sediments, washed free of sediments, and placed in small plastic bags.

In the laboratory, the loosely attached epiphytes were dislodged from the macrophyte shoots by vigorously shaking the bags for about 2 min. The shoots ($n = 5$) and epiphyte material ($n = 5$) from each pot were pooled resulting in one shoot and one epiphyte sample per pot. The shoots were washed three times with deionized distilled water and the resulting suspensions were immediately passed through a 100-μm Nitex mesh screen to separate macrophyte fragments and organisms, e.g., invertebrates. The material passing through the Nitex screen was then concentrated by centrifugation (10 min at 2500 rpm). The resulting supernatant was filtered through a membrane filter (Millipore, 0.45 μm) and the collected material was pooled with the preconcentrated pellet, obtained by the centifugation procedure, prior to drying. The shaking procedure would not have removed tightly attached epiphytic material from the macrophyte shoots (Cattaneo and Kalff 1979) but would prevent the loose epiphytes collected from being contaminated with macrophyte tissue, something that could not have been avoided if we had scraped the shoots.

### Metal Analyses

The cold, or stable (nonlabelled), Co and Mn concentrations were estimated for control and experimental sediments, roots, shoots, and epiphytes. All labware was acid washed (10% HCl) and rinsed a minimum three times in double-distilled deionized water. All acids used were high grade (Analar grade; BDH Inc.). To assess digestion efficiencies and possible contamination, we subjected Buffalo River sediment, citrus leaves, and bovine liver (NBS reference materials Nos. 2704, 1572, and 1577a, respectively) to our laboratory protocol. Concentrated $HNO_3^-$ digested the plant material and dilute aqua regia (3:3:1 $HCl–H_2O–HNO_3^-$) digested the sediment material with 100% efficiency for Co and Mn. We did not analyse samples for total Cs and Eu.

Dried sediments and dried plants were digested with an acid to dry weight ratio of 15 mL:1 g in teflon tubes at room temperature (approximately 20°C) overnight, heated to 90 ± 5°C for 2 h, and allowed to cool. Cooled suspensions were brought to 25 mL final volume in polyethylene volumetric flasks, spun at 2800 rpm for 10 min to remove suspended solids, then analysed for Co and Mn by flame atomic absorption spectroscopy (Perkin Elmer model 5100). Element concentrations of sediments and plants are reported as micrograms per gram dry weight. We combusted, at 550°C, subsamples of the dried epiphyte material and assumed that the loss on ignition represented organic material of the epiphyte matrix. We further assumed that organic material was 50% C and reported epiphyte concentrations as micrograms per gram dry weight.

The hot (labelled) $^{60}$Co, $^{137}$Cs, $^{152}$Eu, $^{154}$Eu, $^{155}$Eu, and $^{54}$Mn concentrations in dried sediments, roots, shoots, and epiphytes were determined by $\gamma$-spectroscopy with a high-resolution Ge detector coupled to a PC-based multichannel analyser. Measured count rates were converted to activities using the counting efficiency (calibrated against known standards), decay, and sample geometry. Values are reported as becquerels per gram dry weight, with the epiphyte weights converted to C as outlined above.

Fully labelled *M. spicatum* was produced by growing plants from 6-cm sprigs to harvesting in the radiolabelled sediments. We assumed the labels were homogeneous between different internal plant element pools. We calculated the specific activities of the sediments, roots, shoots, and epiphytes as, e.g., $^{60}$Co activity/total Co. The percent element in the macrophytes derived from the sediments and the percent element in epiphytes derived from the host macrophyte were calculated as the ratio of the specific activities of the respective components. We used analysis of variance (ANOVA) to test for differences in mean values of measured parameters.

## Results and Discussion

That all radioisotopes detected in the Ottawa River sediments were measured in the experimental *M. spicatum* roots and shoots, but were undetectable in the control sediments, roots, and shoots, demonstrates Co, Cs, Eu, and Mn movement from the sediments into the macrophytes (Fig. 1). We detected radionuclides only in epiphytes growing on experimental plants; none were detected in epiphytes growing on the control plants. These results strongly suggest that the route of element movement is from the sediments through the roots to the shoots and from the shoots to the epiphytes, rather than from the sediments through the water column to the shoots and epiphytes, since the controls, typically growing within 10 cm of the experimental pots, contained no radionuclides. The environment in which the macrophytes were growing was characterised as being shallow (approximately 2 m depth) and exposed to wave action; therefore, any radionuclide leaving the sediments by diffusion would be rapidly mixed into the water column resulting in relatively equivalent exposures for both experimental and control plants.

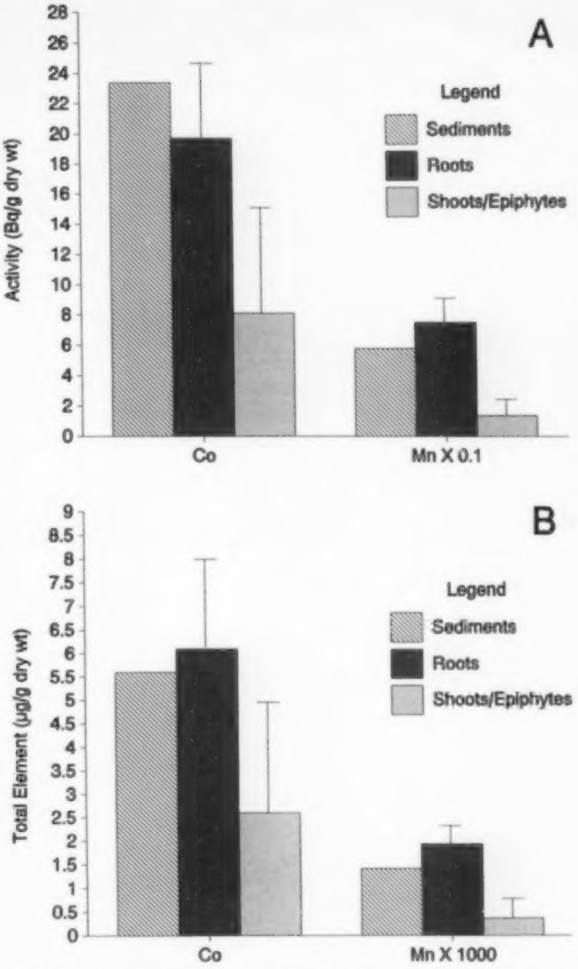

FIG. 1. (A) Activity and (B) total element concentration of Co and Mn in sediments, roots, and shoots and loosely attached epiphytes of *M. spicatum*. Error bars represent 2 SE. No radionuclides were detected in plants or epiphytes on plants grown in uncontaminated sediments (data not shown).

Through ANOVA, we found no statistically significant ($p < 0.05$) differences within elements between sampling dates for root, shoot, or epiphyte to sediment BAFs and hence pooled the data over time. We then tested and found no significant differences in BAFs between roots, shoots, and epiphytes. We pooled the shoots and epiphytes and found significant differences between the pooled samples and the roots for $^{60}$Co ($p < 0.015$) and $^{54}$Mn ($p < 0.001$). For both elements, the roots had significantly higher BAFs (about 1) than for the pooled shoots and epiphytes when compared with the sediments (Table 2). This is most likely due to adsorption or precipitation on the root surface of $^{60}$Co and $^{54}$Mn, the most redox-sensitive radionuclides in our sediments.

Further support for the adsorption hypothesis was found through ANOVA of BAFs between elements within roots, or shoots and epiphytes. There is no statistically significant difference in BAFs between the radioisotopes ($^{60}$Co, $^{54}$Mn,

TABLE 2. Average ($n$ = 7; ±2 SE) root to sediment and shoot and epiphyte to sediment bioaccumulation factors for *M. spicatum* and its epiphytes grown in contaminated sediments in Lake Memphremagog (Quebec–Vermont). The control sediments, roots, shoots, and epiphytes contained no measurable radionuclide activities. Values within a column with different superscripts are significantly different ($p < 0.05$); asterisks indicate significant differences between columns.

| Radioisotope | Root/sediment BAF | Shoot and epiphyte/sediment BAF |
|---|---|---|
| $^{60}$Co | 0.832 ± 0.203$^{a}$* | 0.357 ± 0.308$^{e}$* |
| $^{137}$Cs | 0.152 ± 0.089$^{b}$ | 0.332 ± 0.265$^{e}$ |
| $^{152}$Eu | 0.312 ± 0.070$^{c}$ | 0.337 ± 0.042$^{e}$ |
| $^{154}$Eu | 0.359 ± 0.074$^{c}$ | 0.299 ± 0.178$^{e}$ |
| $^{155}$Eu | 0.459 ± 0.156$^{c}$ | 0.309 ± 0.189$^{e}$ |
| $^{54}$Mn | 1.237 ± 0.133$^{d}$* | 0.220 ± 0.113$^{e}$* |

TABLE 3. Percent uptake (±2 SE) of Co and Mn by *M. spicatum* from sediments to roots, transfer from roots to shoots, and transfer and from shoots to loosely attached epiphytes after three time periods in Lake Memphremagog (Quebec–Vermont). Plants grown in experimental sediments were placed in the lake on 16 June 1991. The number of pooled samples ($n$) is given.

| Days in lake | $n$ | Sediment/ roots | Roots/ shoots | Shoots/ epiphytes |
|---|---|---|---|---|
| | | *Percent transfer of Co* | | |
| 32 | 1 | 75 | 92 | 101 |
| 66 | 3 | 77.7 ± 2.1 | 88.7 ± 2.1 | 92.6 ± 5.2 |
| 98 | 3 | 77.0 ± 2.9 | 97.3 ± 0.9 | 93.7 ± 1.9 |
| | | *Percent transfer of Mn* | | |
| 32 | 1 | 99 | 95 | 99 |
| 66 | 3 | 94.3 ± 3.0 | 99.7 ± 1.0 | 98.0 ± 1.8 |
| 98 | 3 | 93.0 ± 1.5 | 97.1 ± 2.1 | 96.4 ± 2.3 |

$^{137}$Cs, $^{152}$Eu, $^{154}$Eu, and $^{155}$Eu) in the shoots and epiphytes; however, there is a statistically significant difference in BAFs for these elements in the roots (Table 2). Only the three isotopes of Eu were not statistically different from one another. Thus, the roots most likely contained two pools of $^{60}$Co and $^{54}$Mn: an external pool adsorbed to the root surface and an internal pool. That there were no elemental differences in shoot/sediment BAFs suggests that the transfer of the root internal pool to the shoots and subsequent transfer from the shoots to the epiphytes involves no detectable discrimination.

By comparing the specific activities ($^{60}$Co/total Co and $^{54}$Mn/total Mn) between sediments, roots, shoots, and epiphytes, our results show that *M. spicatum* and its attached epiphytes obtained form 75 to 100% of their Co and Mn, respectively, from the sediments within which the macrophytes were rooted (Table 3). Approximately 75% of the root Co was sediment derived, the roots were the Co source for the shoots, and the shoots were the Co source for the epiphytes as the root to shoot and shoot to epiphyte specific activity ratios were 90–100%. We attribute the less than 100% uptake of Co from the sediments by the macrophyte roots as a methodological artifact of the dilute aqua regia digestion we used because this digestion probably overestimates the bioavailable pool of Co. However, it is possible that 25% of the root Co was water column derived (i.e., uptake by the shoots with subsequent translocation to the roots), although the absence of $^{60}$Co in the control plants' shoots and roots makes this unlikely. Thus the 75% uptake of Co from the sediments to roots is probably a minimum estimate for this study.

Virtually 100% of the root Mn was sediment derived, the roots were the source of shoot Mn, and the shoots were the Mn source for the epiphytes (Table 3). As we did not measure stable Cs and Eu, we cannot quantify their percent transfer from sediment to macrophyte based on specific activities. Nevertheless, the BAFs show that Cs and Eu, which are not essential elements for plant growth, are taken up by *M. spicatum* in a similar manner to Co and Mn. Our results for Mn and Co are consistent with experimental studies considering macronutrient nutrition of macrophytes (McRoy and Barsdate 1970; Barko and Smart 1981) and demonstrate that both essential and nonessential trace elements follow a similar pattern of uptake from the sediments, translocation to above-ground tissues, and transfer to the attached epiphytes. Thus, the finding that epiphytes growing on *M. spicatum* in Lake Memphremagog obtained all their Co and Mn nutrition from their host macrophyte demonstrates that this macrophyte functions as much more than a physical support and is not a neutral substrate for its loosely attached epiphytes. While epiphytes can grow on artificial surfaces (Burkholder and Wetzel 1990; Carignan and Kalff 1979), our results demonstrate that when natural plants are the substrate the epiphytes can obtain up to 100% of their trace elements from the host plant.

Two hypotheses could explain our findings that all Co and Mn was supplied to the epiphytes by the host plant. First, the water column concentrations of Co and Mn could be so low that uptake, if any, from the water column might not be detected with our sampling protocol and we would measure 100% uptake from the host plant. In this case the relative uptake from the water or macrophyte might simply reflect the relative concentrations of these two potential sources (our results, however, do not simply reflect elevated concentrations of Co and Mn in the macrophytes because the total Co and Mn concentrations in the experimental and control plants were not statistically different). This would result in the macrophyte being the overwhelming trace element source to the epiphytes. An alternative hypothesis is that the metals released by the host macrophyte are considerably more available to the epiphytes than those metals dissolved in the water column, and thus the water column contribution is small compared with the macrophyte contribution, and not proportional to the total concentration in the respective pools.

We did not directly attempt to determine whether or not the trace elements supplied by the macrophytes would be sufficient to meet epiphyte growth demands. However, since a large component of the loosely attached epiphyte matrix is biotic, a first approximation to examining supply and demand would be to consider the Co and Mn cell quotas for the epiphytes and compare these with the few published data for phytoplankton. We estimated the Mn cell quota of the marine diatom *Thalassiosira pseudonana* by taking a volumetric Mn cell quota (Sunda and Huntsman 1983) and dividing it by an estimated average cell volume of 50 μL (Smayda 1978) to obtain 25 μmol Mn/mol C. Our calculation for the epiphytes is 30 μmol Mn/mol C. Similarly, our estimated cell quota of 4.5 μmol Co/mol C is almost identical to an estimate of 3.06 μmol Co/mol C for the marine diatom *Thalassiosira weissflogii* (Price and Morel 1990). Thus, while the loose epiphyte matrix is composed of bacteria and inorganic material in addition to benthic algae, it appears at first glance that the Co and Mn supplied by the macrophytes to their epiphytes could supply balanced growth if our comparison of the epiphyte matrix with marine phytoplankton is valid.

There is a growing appreciation for the role that rooted aquatic macrophytes play in the biogeochemical cycling of elements in lakes (Lie 1979; Carpenter and Lodge 1986; Jackson et al. 1994). For example, during senescence, *M. spicatum* supplied sufficient N and P to lake waters to affect chlorophyll concentrations (Landers 1982), and weedbeds supplied an amount of P equivalent to 59% of the annual P load to Lake Wingra (Prentki et al. 1979). Although the cycling of sediment-derived trace elements (Al, Cu, Cr, Fe, Mn, Ni, and Zn) by *M. spicatum* has been shown to be small (Jackson et al. 1994), macrophytes do have the potential to make significant contributions to essential and nonessential trace element mass balances in very weedy lakes or in lakes contaminated by pulse inputs. For example, one would expect that the radiocesium introduced into northern European lakes by the Chernobyl accident would remain elevated in macrophytes and epiphytes long after concentrations in the water had dropped because the buried pulse of $^{137}$Cs preserved in the lake's sediments would be in the rooting zone of many macrophytes for many years to come. This scenario would also apply to toxic metals and other contaminants released by industrial accidents. Thus, the sediments represent a large reservoir of both essential and nonessential elements, and contaminants, that could be involved in internal cycling, especially during plant senescence. The transfer of nonessential elements and contaminants from the macrophytes to their epiphytes represents a net input to the littoral zone biota, since these substances are ultimately derived from the sediments, and contaminants in littoral sediments are indirectly available to littoral biota via herbivory or grazing (Fassett 1957; Cattaneo 1983; Lodge 1988) of the epiphytes.

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# Determinants of Postmolt Size in the American Lobster (*Homarus americanus*). II. Folding of Premolt Cuticle

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Cheng, J.-H., and E.S. Chang. 1994. Determinants of postmolt size in the American lobster (*Homarus americanus*). II. Folding of premolt cuticle. Can. J. Fish. Aquat. Sci. 51: 1774–1779.

The effects of eyestalk ablation, regeneration, and rearing condition on the size of new cuticles before and after expansion were studied in juvenile *Homarus americanus*. Both eyestalk-ablated and intact lobster grew larger in large spaces than in small spaces. In contrast, regeneration reduced molt increment. Despite large differences in molt increment (percentage of size increase during a molt) among experimental groups, the percentage of postmolt size increase that could be accounted for by unfolding of the new cuticle was large and constant in each group. In addition, transferring freshly molted lobster to dilute seawater did not produce any further size increase but instead caused cuticle breakage in some animals. We conclude that molt increment in lobster is determined by regulating the size of the cuticle before ecdysis. The size of the postmolt cuticle is primarily a result of unfolding of the new, previously folded cuticle.

On a étudié les effets de l'ablation du pédoncule oculaire, de la régénération et des conditions d'élevage sur la taille de la nouvelle cuticule avant et après le gonflement chez de jeunes *Homarus americanus*. La taille des homards au pédoncule enlevé et des homards intacts s'est accrue davantage dans les grands espaces que dans les petits espaces. Par contre, la régénération a réduit l'accroissement au moment de la mue. Malgré les différences importantes d'accroissement au moment de la mue (en pourcentage) chez les groupes expérimentaux, le pourcentage d'augmentation de la taille après la mue que pouvait permettre la nouvelle cuticule était important et constant dans chaque groupe. En outre, le transfert de homards fraîchement mués dans de l'eau de mer diluée n'a produit aucune augmentation supplémentaire de la taille et a même causé la rupture de la cuticule chez certains. Nous concluons que l'augmentation de la taille au moment de la mue chez le homard dépend de la régulation de la taille de la cuticule avant l'ecdysis. La taille de la cuticule après la mue résulte principalement du dépliement de la nouvelle cuticule.

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Molt increment, the percent increase in size that occurs during each molt cycle, varies according to both intrinsic and extrinsic factors in crustaceans. These include body size, sex, rearing space, limb regeneration, food supply, and eyestalk ablation (see Hartnoll 1982 for review). Although limited intermolt growth can occur in some crustaceans through the extension of the flexible arthrodial membranes linking the exoskeletal plates (Mauchline 1973; Childress and Price 1978), increases in the size of mineralized segments of the exoskeleton occur only at ecdysis when the old cuticle is shed and the new one has not yet hardened. Size increases at ecdysis result from expansion of the unhardened integument. This expansion is caused by increased hydrostatic pressure created by rapid water uptake. The degree of expansion is directly related to the amount of water that is taken up (Drach 1939; Travis 1954; Mykles 1980; deFur et al. 1985; Cheng and Chang 1991a).

Although the mechanisms that ultimately control cuticular expansion at ecdysis are not fully known in crustaceans (see Mantel and Farmer 1983 for review), it does not appear that increases in size at ecdysis depend on the osmotic gradient between surrounding medium and hemolymph (Templeman 1936; Kurata 1962; Haefner and Schuster 1964; Tagatz 1968; Lockwood and Andrews 1969; Knowlton 1974; Hartnoll 1978). This observation appears valid, although the relative importance of the various pathways of water uptake is different when animals molt in different salinities (Cheng and Chang 1991a). These results suggest either that animals take up the precise amount of water they need or that the amount of water that can be retained is limited by the size and extensibility of the new cuticle.

In insects, cuticular inflation at ecdysis, which is caused by swallowing air, involves unfolding of the epicuticle with unfolding or stretching of the exocuticle (see Reynolds 1980 for review). Although the relative contributions of exocuticular unfolding and stretching per se remain to be fully elucidated, there are examples where unfolding alone can account for all the observed increases in length of the next instar (Bernays 1972; Reynolds 1980). Other observations indicate that increases in size of the cuticle can be attributed solely to stretching per se (Bennet-Clark 1962; Cottrell 1962a). Because the epicuticle is inelastic (Bennet-Clark 1963; Filshie 1976), this layer limits the extent to which the cuticle can be expanded. Expansion is not limited by the underlying exocuticle, which briefly becomes reversibly plastic at ecdysis in the blowfly (Cottrell 1962b) or after feeding in *Rhodnius prolixus* (Bennet-Clark 1962). As a result, the size attained after either ecdysis or feeding is determined by the degree of epicuticular unfolding.

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In crustaceans, folds have been observed in the new cuticle of premolt animals (Aiken 1973; Arsenault et al. 1984; Powell and Halcrow 1985). There are, however, no published quantitative data on degree of folding, and size increase at ecdysis was often assumed to involve stretching. In this study, the effects of eyestalk ablation, limb regeneration, and rearing conditions on the size of the new cuticle before and after molt were studied in juvenile American lobster (*Homarus americanus*). The sizes of the new cuticle, before and after expansion, were compared to test the hypothesis that ultimate postmolt size of a lobster is a result of unfolding of the previously synthesized new cuticle.

## Materials and Methods

### Maintenance of Animals

Full-sibling juvenile lobster were raised in semirecirculating systems at 20°C (Chang and Conklin 1993; Conklin and Chang 1993). They were blotted dry, weighed (3–5 g wet weight), and measured with calipers (16.5–19.5 mm in carapace length). They were fed ad libitum with live adult brine shrimp. All experimental animals were placed in the same system.

### Treatments

Various treatments were used to amplify the variation in postmolt size.

*Limb removal*

Lobster were induced to autotomize both of their chelipeds by applying constant pressure to each merus. In one experiment, regenerating cheliped limb buds were further removed in some animals using the same method. Chelipeds were regenerated during the same molt cycle only in those animals that had limbs or limb buds removed (induced to autotomize) in stages earlier than stage $D_1^3$ (Cheng and Chang 1993a).

*Eyestalk ablation*

To produce eyestalk-ablated (ESX) lobster, both eyestalks were removed at their bases from intact animals at molt stage C–$D_0$ using a pair of fine scissors. Survival was approximately 90%.

*Rearing space*

Lobster at molt stage C–$D_0$ were raised in trays divided into either large (190 × 190 mm) or small squares (95 × 95 mm). Lobster were raised in small squares if not specified.

*Osmotic shock*

To determine if the new cuticle could be further stretched by increasing the internal pressure, lobster were transferred to dilute (60 or 75%) seawater within 1 h after ecdysis.

For all treatments, body weight was measured to the nearest 10 mg and carapace length (CL) and cheliped (propus) width (CW) were measured to the nearest 10 μm using calipers before treatment and 1 wk after molt. The percent increase in these parameters was calculated based on the following equation:

$$[(X_a/X_b) - 1] \times 100$$

where $X_a$ and $X_b$ are either CL or CW measured from the same lobster after and before molt, respectively. Exopodites of the fifth pair of pleopods (pleopod width; PW) were measured to the nearest 10 μm using a dissecting microscope equipped with a micrometer.

### Measurement of Size of New Cuticle before and after Postmolt Expansion

The exopodites of the right fifth pair of pleopods were dissected from animals expected to exuviate within 7 h. This is when the last distinct premolt stage prior to ecdysis occurs (Cheng and Chang 1991b). It is dependent on the degree of cuticle digestion along the dorsal midline of the carapace and on the dorsal surface of the merus of the chelipeds. Samples were fixed in a solution containing 2.5% glutaraldehyde, 670 mM glucose, 10 mM $CaCl_2$, and 100 mM sodium cacodylate (pH 7.2) for 4 h and postfixed in a solution containing 1.0% osmium tetroxide, 750 mM sucrose, and 100 mM sodium cacodylate (pH 7.2) for 2 h. They were dehydrated with an acetone series and embedded in low-viscosity resin (Spurr 1969). Thick (1 μm) transverse sections from the midregion of the pleopods were stained with 1% toluidine blue and photomicrographs were made of each section.

The outlines of both cuticles (the outer old and the inner new) were traced from the photographic print (final magnification of approximately 200×) onto tracing paper. The perimeters of both cuticles were measured from tracings with the aid of computerized image analysis (C-2 Image Analyzer, Olympus Corp.). These data were used to calculate predicted molt increment. The observed molt increment was calculated based on the width of the contralateral pleopod measured in situ on the same lobster 1 wk before and after ecdysis.

Statistical analysis was performed with the aid of a SAS-GLM program (SAS Institute Inc.) designed for the analysis of variance with uneven sample sizes. The comparison among treatment groups was made using Duncan's multiple range comparison test (after ANOVA). All comparisons were made at a significance level of 0.05. All values are expressed as means ± 1 SD. In Tables 1–3, means in each column followed by the same letter are not significantly different ($p > 0.05$). Each group contained at least seven animals.

## Results

### Rearing Space and Eyestalk Ablation

The effects of rearing space and eyestalk ablation on percent postmolt increase in carapace length (%CL) and cheliped width (%CW) were investigated (Table 1). Both ESX and intact lobster grew better in large versus small spaces. While intact lobster grown in large spaces had larger increases in %CL (4.2%; 18.2 minus 14.0%) and %CW (5.0%) than those grown in small space, ESX lobster had corresponding values of only 2.2%CL and 3.7%CW.

In small spaces, ESX lobster had higher %CL's than intact controls. In large spaces, however, ESX lobster had the same %CL as intact ones. In either small or large spaces, the differences in %CW's were much larger than in %CL's between ESX and intact lobster. Thus, the ratios of allo-

TABLE 1. Effects of eyestalk ablation and rearing conditions on carapace length (CL) and cheliped width (CW) in lobster.

| Experimental group | Percent increase in CL | Percent increase in CW | Ratio of percent increase of CW to CL | *n* |
|---|---|---|---|---|
| Small space | | | | |
| Ablated | 16.2 ± 0.5 *b* | 30.5 ± 2.3 *a* | 1.88 *a* | 6 |
| Intact | 14.0 ± 0.9 *c* | 18.3 ± 1.9 *c* | 1.31 *b* | 9 |
| Large space | | | | |
| Ablated | 18.4 ± 1.8 *a* | 34.2 ± 4.7 *a* | 1.86 *a* | 7 |
| Intact | 18.2 ± 1.5 *a* | 23.3 ± 4.1 *b* | 1.28 *b* | 9 |

TABLE 2. Effects of cheliped and limb bud regeneration or loss on carapace length (CL) and cheliped width (CW) in lobster.

| Experimental group | Percent increase in CL | Percent increase in CW | Ratio of percentage increase of CW to CL | *n* |
|---|---|---|---|---|
| Chelipeds intact | | | | |
| Normal size | 14.2 ± 1.5 *a* | 19.4 ± 2.2 *a* | 1.36 ± 0.13 *a* | 22 |
| Newly regenerated[a] | 13.4 ± 0.9 *b* | 33.4 ± 4.0 *b* | 2.50 ± 0.24 *b* | 27 |
| Chelipeds removed | | | | |
| Nonregenerated[b] | 12.6 ± 1.4 *b* | — | — | 15 |
| Regenerated[c] | 8.8 ± 0.9 *c* | — | — | 27 |
| Limb buds removed | | | | |
| Nonregenerated[d] | 8.2 ± 0.6 *c* | — | — | 11 |
| Regenerated[e] | 9.8 ± 1.3 *d* | — | — | 18 |

[a] Newly regenerated chelipeds were 15% smaller in size than normal ones.
[b] Chelipeds were autotomized at stage $D_1^3$.
[c] Chelipeds were autotomized before stage $D_1^2$.
[d] Regenerating buds of chelipeds were autotomized at stage $D_1^3$.
[e] Regenerating buds of chelipeds were autotomized before stage $D_1^2$.

metric growth were much higher in ESX lobster (1.86–1.88) than in intact controls (1.28–1.31) (Table 1). In ESX lobster, the increased ratio of allometric growth in cheliped width was accompanied by increased body weight gain after molt compared with controls (data not shown).

### Limb Regeneration

The effects of regeneration on %CL and %CW were also investigated (Table 2). Regeneration reduced %CL by 5.4% compared with intact controls. The reduction in %CL was only 1.6% for lobster whose chelipeds were autotomized but not regenerated. In a further attempt to alter %CL's, primary limb buds were removed (through induced autotomy) from regenerating lobster. No further decreases in %CL's were noted whether or not the lost primary limb buds regenerated before the ensuing molt (8.8 versus 8.2 and 9.8%; Table 2).

Newly regenerated chelipeds were 15% smaller than normal; however, a compensatory growth occurred during the subsequent molt. As shown in Table 2, the ratio of allometric growth in cheliped width was 2.50 in lobster with newly regenerated chelipeds, which is almost twice the value (1.36) found in lobster with normal, nonregenerated chelipeds. As a result, this deficit of 15% in the width of newly regenerated chelipeds then diminished to only 5% following the next molt (second postautotomy molt).

### Cuticular Folding

Pleopods were chosen to study the relationship between cuticular folding and molt increment because of their size, shape, and thin cuticle. Lobster were divided into four groups. Each group received a different treatment (eyestalks intact in large spaces, ESX in small spaces, eyestalks intact in small spaces, and regenerating limb buds in small spaces). These treatments widened the possible range of the resulting molt increments (Table 3).

To determine if the removal of the right pleopod affected the size increase in the left contralateral, pleopod samples were not taken from some control lobster. In each experimental group, there were no significant differences between these two subgroups in the percent increase in either carapace length or left pleopod width. These results indicated that pleopod sampling did not affect postmolt size increase.

A representative cross section of the right exopodite of the fifth pair of pleopods prepared from a lobster approximately 7 h before ecdysis (Fig. 1) shows that new cuticle has undergone substantial synthesis, while the old one displays extensive digestion. The outline of the old cuticle is smooth, while that of the new one has many folds. The perimeters of both cuticles were used to calculate the predicted percent increase in the pleopod width (%PW). The predicted %PW's followed the observed values closely (Table 3). Interestingly, the differences between the two

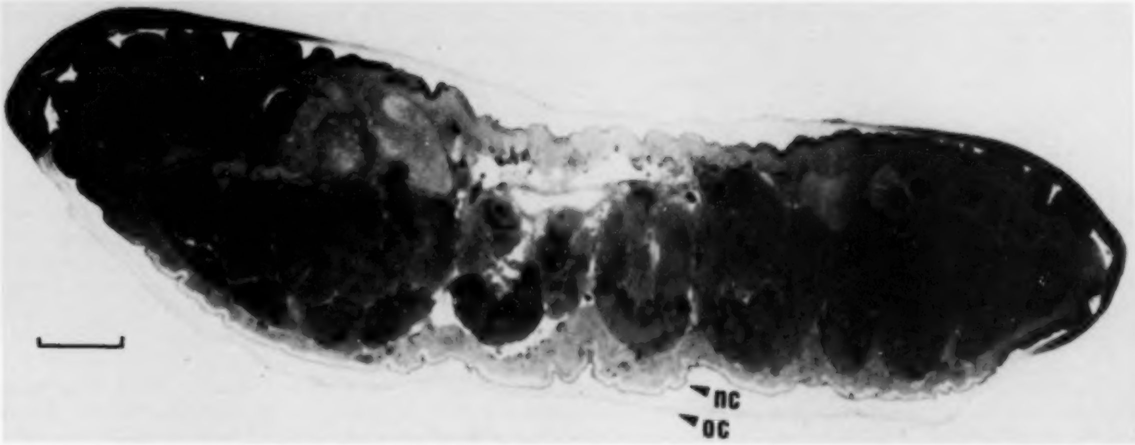

FIG. 1. Cross section of a pleopod dissected from a lobster approximately 7 h before ecdysis showing the contours of both the old (oc) and new (nc) cuticles. Bar represents 100 μm.

TABLE 3. Relationship of cuticular folding and postmolt size in the percent increase in pleopod width (%PW) in lobster.

| Treatment | Observed %PW[a] | Predicted %PW[b] | Percent difference[c] | *n* |
|---|---|---|---|---|
| Large space | | | | |
| Intact | 16.8 ± 2.3 *a* | 12.7 ± 3.3 *a* | 4.1 ± 5.6 *a* | 14 |
| Small space | | | | |
| Ablated | 16.5 ± 2.3 *ab* | 12.3 ± 3.2 *a* | 4.2 ± 5.5 *a* | 11 |
| Intact | 14.9 ± 2.6 *b* | 10.5 ± 2.7 *a* | 4.4 ± 5.3 *a* | 14 |
| Claw removal | 7.1 ± 3.4 *c* | 2.6 ± 3.4 *b* | 4.5 ± 6.8 *a* | 10 |

[a] Observed %PW's were calculated using the left pleopod widths before and after molt.

[b] Predicted %PW's were calculated using the perimeters of the new and old cuticle on the same cross section of the right pleopod prepared approximately 7 h before ecdysis.

[c] Difference between observed and predicted values.

were about the same in each experimental group (4.1, 4.2, 4.4, and 4.5%).

When placed into hypoosmotic (60 or 75%) seawater within 1 h postmolt, lobster did not show any significant additional increases in either carapace length or body weight (Table 4). Instead, four lobster, two each in the 60 and 75% seawater, showed signs of cuticle rupture in either the propus of the cheliped or the branchial region of the carapace. Of these four lobster, three died and the sole survivor had a large scar on the cheliped propus.

## Discussion

Koshio et al. (1989) reported that ESX lobster weighed more than intact controls of the same carapace length. In light of our data, their observation could be explained by the exaggerated increase in cheliped weight. Changes in allometric growth in ESX animals have also been observed in *Uca pugilator* (Hopkins 1985). But, in contrast with lobster, the ratio between cheliped and carapace lengths decreased in ESX crab. In lobster, the enhanced allometric growth of chelipeds was also found in the subsequent growth of newly regenerated chelipeds (Table 2), in cheliped transformation (Govind and Pearce 1989), and in sexual differentiation (Aiken and Waddy 1989).

The excessive swelling at ecdysis found in ESX *H. americanus* has also been observed in many other ESX crustaceans (see Muramoto 1988 for review). This phenomenon was partially reversed if the ESX animals were implanted with sinus glands (Scudamore 1947), given injections of sinus gland extracts (Carlisle 1956), or reimplanted with eyestalks, brains, or thoracic ganglia (Bliss et al. 1966). A specific eyestalk hormone controlling the amount of water uptake at ecdysis was therefore suggested (Carlisle 1956; Knowles and Carlisle 1956; Bliss et al. 1966; Mantel et al. 1975). Direct evidence, however, of a specific eyestalk hormone that controls water uptake is still lacking. Furthermore, the above hypothesis does not explain the observation that the effects of eyestalk ablation largely disappeared if the operations were made on crab or lobster after a critical stage (Passano 1953; Cheng and Chang 1993a) or if both ESX and intact lobster were raised in a more favorable environment (Table 1).

Passano (1960) provided an alternative explanation for the abnormal size increases of ESX crab. He proposed that the integument formed in ESX animals is more elastic than

TABLE 4. Effect of salinity on postmolt body weight gain and carapace length (CL) increase in lobster.

| Percent seawater | Percent increase in body weight | Percent increase in CL | n |
|---|---|---|---|
| 100 | 36.5 ± 5.9 | 12.7 ± 1.8 | 8 |
| 75 | 40.9 ± 4.3 | 13.4 ± 1.1 | 8 |
| 60 | 37.4 ± 4.3 | 13.5 ± 0.8 | 6 |

in intact animals. Green and Neff (1972) reported that the structure of the cuticle of newly molted ESX crab (*U. pugilator*) is slightly different from that of intact animals in that the lamellae are less tightly organized. There is no direct evidence, however, to support the hypothesis that ESX animals have a more elastic cuticle.

In our study, excess growth of the pleopods in ESX lobster can be explained by the increased size of the folded new cuticle. This observation does not support the above hypotheses that ESX animals take up more water at ecdysis because (1) they produce more elastic cuticle or (2) they lack a specific eyestalk hormone capable of regulating the amount of water uptake at ecdysis. In large spaces, intact lobster expressed larger molt increments compared with those in small spaces. Again, these results are supported by observations of larger new cuticles synthesized prior to expansion.

Although both eyestalk ablation and large space resulted in larger increases in molt increment, the cellular mechanisms that underlie it are different. While eyestalk ablation caused an increase in the area of cuticle produced by each individual epidermal cell, large space treatment resulted in an increase in the number of epidermal cells (Cheng and Chang 1993b).

In lobster, as in other decapods, regeneration of chelipeds reduced the molt increment of other body parts (see Skinner 1985 for review). The degree of reduced growth depends on the total mass of the lost appendages (Skinner and Graham 1972; Kuris and Mager 1975; Hopkins 1985). The concept of regeneration load has been applied to this phenomenon (Skinner and Graham 1972). It has been suggested that the decreased molt increment following regeneration is a result of a redistribution of water taken up at ecdysis to inflate the regenerated appendages (Hopkins 1985). However, the observation that removal of limb buds at late premolt stages ($D_1^3$–$D_3$) did not further alter postmolt size in both lobster (this study) and crab (*Hemigrapsus oregonensis*; Kuris and Mager 1975) does not support the above suggestion. Instead, reduced growth in regenerated arthropods may be a result of energy partitioning between regeneration and normal body growth (Stock and O'Farrell 1954). Our observations that regenerated lobster had the smallest molt increment and smallest size of new cuticle before expansion indicate that regenerated lobster decreased molt increment by reducing the size of cuticle synthesized during late premolt of the preceding molt cycle. Our data also support the hypothesis of Kuris and Mager (1975) that regenerated crab may reduce the molt increment by reducing the frequency of cell division occurring in early premolt of the preceding instar. Their contention is further confirmed by our observation that the area of cuticle produced by each individual epidermal cell was constant (Cheng and Chang 1993b).

In this study, the size of the new cuticle prior to ecdysial expansion was 4.1–4.5% less than the size of it after expansion. This indicates that lobster regulate most of their postmolt size by regulating the size of the new cuticle produced prior to molt. Although the above differences suggest that a small amount of stretching may exist, the observation that cuticles broke without any further increase in size following administration of excess internal osmotic pressure (due to transfer to dilute seawater) indicates that cuticles are inelastic. In addition, the observations that various manipulations to increase cuticular size (eyestalk ablation or variations in living space) had to be made before, and not after, epicuticle was synthesized (Cheng and Chang 1993a) imply that it is size of the epicuticle that is important in determining the postmolt size increase in lobster.

The inelasticity of the epicuticle seems to be a general feature of most arthropods. The first layer of the epicuticle–cuticulin portion of the exoskeleton is probably present in all arthropods and covers the entire body surface except certain sense organs and the midgut (Filshie 1982; Locke 1984). The inelasticity of the epicuticle defines the size and shape of the animals.

The small portion of the postmolt increase in pleopod size observed in our study that cannot be explained by unfolding of new cuticle may represent microfolds of new cuticle that were not measurable. In the locust (*Schistocerca gregaria*), pharate cuticle shows macrofolding as well as microfolding. While macrofolds are large enough to extend throughout the entire depth of the cuticle, microfolds are not. Although most of the size increase at molt comes from the extension of macrofolds, unfolding of microfolds may contribute significantly to size increase at ecdysis (Bernays 1972).

Salinity did not affect molt increment, even if animals were transferred into dilute seawater before their cuticles were fully expanded. This result is consistent with other published reports (see Hartnoll 1982 for references). Although some lobster died of cuticular breakage, indicating that the internal pressure was too high, most postmolt lobster transferred to low salinity survived without a further increase in body size, suggesting that excess water is released through urine (Cheng and Chang 1991a). Hagerman and Larsen (1977) found that the frequency of urination increased from once per 50–55 min in intermolt to once per 15–20 min in early postmolt. The urination rate also increased from 23.9 $\mu L \cdot g^{-1} \cdot h^{-1}$ to 65.5 μL. The increased urination in early postmolt was believed to reflect increased water entry through relatively higher permeable body surface (Rudy 1966; Lockwood and Inman 1973).

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# A Predation Bioassay to Quantify the Ecological Significance of Sublethal Responses of Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) to the Antisapstain Fungicide TCMTB

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Kruzynski, G.M., and I.K. Birtwell. 1994. A predation bioassay to quantify the ecological significance of sublethal responses of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to the antisapstain fungicide TCMTB. Can. J. Fish. Aquat. Sci. 51: 1780–1790.

The lumber protection antisapstain fungicide 2-(thiocyanomethylthio)benzothiazole (TCMTB) is leached by rainfall from treated wood awaiting shipment from outdoor lumber storage facilities. Stormwater discharges of this pesticide into estuarine reaches of the Fraser River, British Columbia, have raised concern about toxic effects on juvenile salmon rearing in this habitat. Simulated stream flow conditions were used to expose underyearling chinook salmon (*Oncorhynchus tshawytscha*) to a sublethal (10 $\mu g \cdot L^{-1}$) concentration of TCMTB. Equal numbers of fin-clipped control and exposed fish were subsequently transferred to a vertically stratified (fresh over seawater) 15 500-L outdoor tank where they were challenged with the combined stressors of salinity and the presence of a marine predator (yellowtail rockfish (*Sebastes flavidus*)). After 5 d under these simulated estuarine conditions, survivors were enumerated. Chinook salmon that had been exposed to the toxicant were consumed in preference to the control group by a factor of 5.5:1. Inasmuch as predator avoidance represents the successful integration of appropriate biochemical, physiological, and behavioural responses, the predator bioassay provides an ecologically relevant technique to determine the significance to survival of the complex multifactorial interactions of individually "sublethal" stressors.

Le fongicide anticoloration 2-(thiocyanométhylthio)benzothiazole (TCMTB), qu'on utilise pour traiter le bois entreposé à l'extérieur en attendant son expédition, est lessivé par les pluies. Les eaux pluviales chargées de ce pesticide, qui se déversent dans les biefs estuariens du fleuve Fraser (Colombie-Britannique), ont suscité de l'inquiétude au sujet des effets toxiques éventuels sur les saumons juvéniles se développant dans cet habitat. On a simulé les conditions de courant d'un cours d'eau pour exposer des saumons quinnat (*Oncorhynchus tshawytscha*) de moins d'un an à une concentration subléthale (10 $\mu g \cdot L^{-1}$) de TCMTB. Un nombre égal de témoins aux nageoires rognées et de poissons exposés ont été ensuite transférés dans un bassin extérieur de 15 500 L renfermant une couche d'eau douce sur une couche d'eau de mer, où ils ont été soumis à deux agents stressants : l'eau de mer et la présence d'un prédateur marin (sébaste à queue jaune (*Sebastes flavidus*)). Après 5 jours de ces conditions estuariennes simulées, on a dénombré les survivants. Les saumons quinnat qui avaient été exposés à la substance toxique ont été les victimes du prédateur de préférence aux témoins dans un rapport de 5,5 : 1. Comme l'évitement du prédateur implique l'intégration d'un ensemble de réactions d'ordre biochimique, physiologique et comportemental, l'essai biologique au moyen des prédateurs fournit une technique pertinente du point de vue écologique pour déterminer l'importance pour la survie des interactions multifactorielles complexes stressants « subléthaux » individuels.

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The Fraser River in British Columbia has long enjoyed a reputation as one of the world's major producers of Pacific salmon (*Oncorhynchus* sp.). However, since the 1950's there has been a steady decline in adult returns of chinook (*O. tshawytscha*) and coho salmon (*O. kisutch*) (Fraser et al. 1982). More recently, Starr and Schubert (1990) reported that the Harrison River fall chinook stock, which rears in the Fraser River estuary and constitutes the major contributor to both the Strait of Georgia commercial and sport fishery, has been in a state of virtual "collapse" since 1986. In addition to overfishing, the degradation of habitat and possible sublethal effects of complex mixtures of pollutants in the river are factors which could be contributing to this decline. Offspring of major stocks of fall chinook salmon move seaward within 90 d of hatching and spend from weeks to months migrating and rearing in the shallow surface waters of the Fraser River estuary (Levy and Northcote 1982). During this time, these salmon must adapt to seawater, locate and capture food, and evade predators. Furthermore, because they tend to occupy less saline estuarine surface waters which stratify over deeper salt water (Birtwell and Kruzynski 1989), juvenile salmon are especially vulnerable to the combined impact of pollutants which are discharged into the river.

Although by world standards the Fraser River is not highly industrialized, its contamination by persistent organic xenobiotics has been well documented (e.g., Birtwell et al. 1988; Rogers et al. 1990). Chlorophenols and chloroguaiacols in fish, water, and sediments have been traced to pulp and paper mills discharging effluent hundreds of kilometres upstream and to sawmills located in the lower reaches of the river (Carey 1988; Carey and Hart 1988; Rogers et al. 1988, 1990; Servizi et al. 1988). In addition to discharges from three major municipal waste treatment plants, the estuarine reaches of the Fraser River receive wastes from numerous industrial locations as well as from a multitude of "nonpoint" sources. Not only is the environmental fate of this complex mixture of xenobiotics a subject for concern, but the interactions of contaminants with changing physicochemical conditions characteristic of estuaries are poorly understood. Other than the documentation of contaminant uptake, the sublethal effects of these xenobiotics on organisms utilizing this habitat remain virtually unknown (Birtwell et al. 1988).

Stormwater runoff from lumber preservation industries located on the lower reaches of the Fraser River was found to constitute a major source of tetra- and pentachlorophenol contamination (Krahn et al. 1987a, 1987b). These findings proved to be the stimulus for the promulgation of legislation banning the use of chlorophenates in British Columbia as antisapstain fungicides for the protection of freshly cut lumber. In late 1988, and despite their temporary registration for use, the environmental fate and aquatic toxicology of the replacement chemicals were poorly known, especially so under the application conditions pertinent to coastal British Columbia. One such antisapstain chemical, 2-(thiocyanomethylthio)benzothiazole (TCMTB), replaced the bulk of the market share previously held by chlorophenates. Accordingly, the possibility of TCMTB-laden stormwater discharging into vertically stratified coastal surface waters stimulated a comprehensive research effort on environmental fate and sublethal toxicology of this fungicide by the Department of Fisheries and Oceans and Environment Canada. Krahn (1990) studied the leaching of TCMTB by rainfall from freshly treated lumber at a modern sawmill situated on the lower Fraser River estuary. Stormwater runoff contained 240–340 $\mu g$ TCMTB·$L^{-1}$, and dye studies indicated that the surface effluent plume stratified over more saline waters. The TCMTB concentrated in a plume in the top metre of the water column and moved along the shore with tidal currents, thereby intruding on habitats used by migrating and rearing juvenile salmon. Within the plume, the concentration of TCMTB was from 2 to 10 times the 96-h LC50 (range 6–18 $\mu g \cdot L^{-1}$) for juvenile salmonids (Department of Fisheries and Oceans, unpublished information; MacKinnon 1991).

Whereas the volume of stormwater runoff is simply determined by rainfall, the degree of chemical leaching depends on the efficiency of application, the type of lumber being processed, and the duration of outdoor storage after spray application. For example, Krahn and Shrimpton (1988) reported an average total chlorophenols of 1968 $\mu g \cdot L^{-1}$ in storage yard runoff following high-pressure spray application. Subsequently, Krahn (1990) found 7500 $\mu g$ TCMTB·$L^{-1}$ in runoff from high-pressure-sprayed planed lumber given less than 1 h of storage; this figure dropped to 340 $\mu g \cdot L^{-1}$ after at least 12 h of covered storage prior to exposure to rainfall. It is therefore not uncommon for the range of concentrations of antisapstain chemicals discharged to receiving waters to exceed acutely toxic levels by several orders of magnitude. As a consequence, not only may juvenile salmon be exposed to sublethal concentrations of antisapstain chemicals for extended periods of time, but over shorter intervals they might also have to endure repetitive acutely toxic exposures. In this study, we attempted to determine the ecological significance of such encounters by measuring the vulnerability to predation of chinook salmon after sublethal exposure to TCMTB in the laboratory. To accomplish this, we utilized a behavioural bioassay based upon that designed by Goodyear (1972). We consider the consequences of sublethal effects leading to the death of an animal due to a secondary effect such as predation to be a form of "ecological death" which is neither well researched nor readily understood.

A number of authors have demonstrated the utility of the "ecological death" (Sprague 1971; Vandermeulen 1982) concept in small-scale laboratory aquarium studies. For example, Sylvester (1972) and Coutant (1973) investigated the consequences of sublethal temperature shock, while Kania and O'Hara (1974) studied predator–prey interactions as affected by sublethal exposure to mercury. Larger systems were used by Sullivan et al. (1978) to study the effects of cadmium on predation by largemouth bass (*Micropterus salmoides*), while Finger et al. (1985) modified Sullivan's circular chambers to study predation-induced mortality of bluegill (*Lepomis macrochirus*) exposed to fluorene. Hedtke and Norris (1980) used large laboratory streams to determine the vulnerability to predation of chinook salmon after sublethal exposure to ammonium chloride. Hatfield and Anderson (1972) used 11 000-L outdoor ponds to test the vulnerability to predation of Atlantic salmon (*Salmo salar*) parr after sublethal exposure to insecticides used for aerial spraying. The most recent and largest predator–prey system (15 000-L circular tanks) was used by Olla et al. (1992) to study the effects of handling stress on predator avoidance capabilities of seawater-adapted coho salmon smolts.

In our study, we used a 15 500-L circular outdoor tank in which we stratified freshwater over seawater, thus adding a volitional salinity stress to the experimental design while simulating vertically stratified estuarine conditions. By combining the latent effects of toxicant exposure with the additional stressors of salinity and the presence of marine predators below the halocline, we tested the susceptibility to predation of chinook salmon smolts in seawater after sublethal exposure to the antisapstain fungicide TCMTB in freshwater. This simulation, albeit simplified, is typical of that which is likely to occur in the Fraser River, or other coastal estuaries, and to our knowledge is the first time that such an approach has been taken.

## Materials and Methods

Earlier attempts to test differential predation on TCMTB-exposed and unexposed underyearling salmonids were made using 2-yr-old West Vancouver Laboratory (WVL) reared chinook salmon as predators. These studies, which were conducted in a 4500-L rectangular (Water Column Simulator, WCS) experimental aquarium described by Birtwell and Kruzynski (1987), were generally unsuccessful. Although the chinook salmon had been reared on live salmonid prey in circular 3600-L outdoor holding tanks, prey capture within the angular confines of the WCS was inconsistent. Moreover, their feeding strategy was to attack faster moving rather than lethargic prey. As a result, TCMTB-exposed underyearling salmon, which would characteristically maintain position against the current with minimum lateral excursion, and sought shelter along the walls or in corners of the WCS aquarium, were rendered less accessible to the chinook predator than the normally active or "control" prey. We concluded that a predator with a different feeding strategy should be used. In addition, some natural cover was provided, not only as a refuge for the prey, but also to simulate a more realistic environment where the predator could conceal itself and stalk the prey.

Three independent sets of experiments were carried out. Two of these were conducted indoors using rectangular troughs containing (a) a mixture of marine predators with varying feeding strategies (Pacific staghorn sculpin (*Leptocottus armatus*), great sculpin (*Myoxocephalus polyacanthocephalus*), Dungeness crab (*Cancer magister*), and red rock crab (*Cancer productus*)) and (b) northern squawfish (*Ptychocheilus oregonensis*) as a representative freshwater predator. The present paper describes the rockfish study; the other two studies will be reported elsewhere.

### Rockfish Predators

We used yellowtail rockfish (*Sebastes flavidus*) as a marine predator in a vertically stratified tank. This species is representative of an opportunistic piscivorous midwater predator that probably feeds on prey of the size of juvenile Pacific salmon (Lorz et al. 1983) and lives in coastal areas in the vicinity of reefs, ledges, and kelp beds. The success of the Vancouver Public Aquarium in holding this species in seawater of salinity similar to that pumped to our laboratory (25–28‰) reinforced the selection of the yellowtail rockfish as a predator for our studies.

The rockfish were procured through a commercial collector (Seacology Marine Life Supply, Vancouver, B.C.). Since behavioural experiments were planned, special care was taken to collect and handle specimens in a way that would minimize stress. A number of rockfish were caught on barbless hooks at the entrance to Ucluelet Harbour, Vancouver Island, on March 19, 1991. Four lightly hooked specimens were selected and delivered in a live tank to our laboratory within 24 h of capture. Three of these were placed in a specially designed fibreglass rearing tank in our outdoor aquarium facility.

The cylindrical "predation tank", measuring 3.05 m in diameter and 2.4 m in height, was filled to a depth of 2.1 m with 15 500 L of seawater. Seawater from our laboratory supply was air equilibrated through a packed column (Owsley 1981) and delivered to the tank at approximately 50 $L \cdot min^{-1}$, providing 90% replacement within 12 h (Sprague 1969) and a peripheral circular current in the range of 10–15 $cm \cdot s^{-1}$. Periodic additions of kelp (*Nereocystis* sp.) and natural algal growth on the walls and the bottom of the tank provided cover. However, because we were unable to maintain *Nereocystis* for more than a week under these conditions, artificial "kelp" was added in the form of black polyethylene film fronds anchored to the bottom with rocks. Algal growth soon covered the plastic and the yellowtail rockfish remained motionless among the fronds for extended periods of time.

For 6 wk prior to start of the predation experiments in June 1991, rockfish were fed exclusively on underyearling salmonids (chinook and coho salmon and rainbow trout (*Oncorhynchus mykiss*)). Consumption rates determined during this acclimatization period were used to specify the numbers of prey to be added during the experimental predation trials, so that approximately half would be consumed within 5 d. In addition, the water supply and drainage systems were redesigned so that the tank could be stratified vertically (Birtwell and Kruzynski 1989) with a freshwater lens (0.38 m; 2800 L) separated by a sharp halocline (0.06 m; 440 L) and overlying 1.66 m (12 100 L) of seawater. The predation tank was stratified in the afternoon prior to the initiation of the predation trials which started with the addition of prey at 09:00.

### Prey

Our stock of fall chinook salmon originated from eggs fertilized in October 1991 at the Department of Fisheries and Oceans Chilliwack River Hatchery, B.C. These fish were brought to the WVL on April 11, 1991, and reared on air-equilibrated well water (pH 6.6–6.7; conductivity 220 $\mu S \cdot cm^{-1}$, total hardness 49.8 $mg \cdot L^{-1}$; dissolved oxygen at air saturation) at a flow rate that maintained loadings at 0.35–0.56 $kg \cdot L^{-1} \cdot min^{-1}$. The fish were reared in a 2.44-m-diameter fibreglass tank filled to a depth of 0.75 m, providing a volume of 3500 L.

The water inlet was positioned to establish a circular current so that fish could orientate to and maintain position against the flow; the time for 90% replacement was approximately 4 h. Automatic belt feeders (FIAP, Zeigler Bros. Inc., Pennsylvania) were loaded once daily to provide Oregon Moist Pellet (OMP) at a ration of 2% body $weight \cdot d^{-1}$ except for the 24-h period which preceded handling. Feed was made available from dawn to dusk to avoid behavioural entrainment on routine hatchery feeding schedules and special care was taken to minimize disturbance. At the time of experimentation, the chinook salmon were 8–9 mo old, had reached a fork length of 90–100 mm and a weight of 6–9 g, and appeared to have smolted successfully as evidenced by coloration and the ability to tolerate direct transfer to seawater of 27–29‰.

### Handling

All transfer procedures requiring anaesthesia were conducted using an approximately isosmotic mixture of well water and seawater (2:1) to mitigate hydromineral disturbance and reduce stress due to handling (Wedemeyer 1972; Long et al. 1977; Haux and Sjobeck 1985). This procedure also served to raise the pH of our well water, and by increasing buffering capacity was designed to minimize the pH-related stress due to anaesthesia with MS-222 (Wedemeyer 1970).

Batches of approximately 20 chinook salmon were netted from the stock rearing tank into a darkened bucket containing 20 mg MS-222$\cdot L^{-1}$ in preparation for fin clipping. Lightly anaesthetized fish were quickly removed, right or left pelvic fin clipped, and then allowed to recover in buckets containing aerated brackish water. A few minutes later, the fish were transferred to annular "exposure" tanks (described below) for acclimation, where they quickly took up position against the current. Thus the start of the acclimation began within approximately 20 min of initial capture in the stock rearing tank. Feeding was resumed the following day using automatic circular platform feeders (Nagel 1974) which delivered 3/32 OMP from dawn to dusk at a ration of 2% body $weight \cdot day^{-1}$.

TABLE 1. Comparison of water quality between well and municipal dechlorinated water at West Vancouver Laboratory, June–July 1991 (mean values of data from water samples collected on June 7 and July 2, 1991).

| | Well | Municipal |
|---|---|---|
| Na ($mg \cdot L^{-1}$) | 23.4 | 0.5 |
| K ($mg \cdot L^{-1}$) | 1.7 | 0.2 |
| Ca ($mg \cdot L^{-1}$) | 17.6 | 1.3 |
| Cl ($mg \cdot L^{-1}$) | 45.9 | 1.7 |
| Alkalinity ($mg \cdot L^{-1}$) | 28.0 | 0.9 |
| Total hardness ($mg \cdot L^{-1}$) | 60.2 | 3.8 |
| Conductivity ($\mu S \cdot cm^{-1}$) | 251.0 | 14.0 |
| pH | 6.8 | 6.0 |

A period of 96 h was provided to ensure recovery from the stress of capture, anaesthesia, and handling before starting the toxicant exposure (Miles et al. 1974; Soivio and Oikari 1976; Swift 1981; Pickering et al. 1982). After the acclimation period, the designation of "control" and "exposed" tanks was made at random. Although this resulted in TCMTB-exposed fish in both trials having left pelvic fins clipped, ancillary experiments indicated that clip location had no bearing on susceptibility to predation under our experimental conditions.

Control and TCMTB exposures were conducted under simulated stream flow conditions in 70-L annular fibreglass tanks (Kruzynski 1979) 89 cm OD, 51 cm ID, 19 cm deep, and filled to a volume of 60 L. By adjusting the output of recirculating pumps, the water velocity was set at 1.5 body lengths$\cdot s^{-1}$. A shaded area was provided for cover upstream of the toxicant delivery point. A simple Mariotte bottle system (Burrows 1949), as modified by Leduc (1966) to facilitate fine adjustment, was used to meter the TCMTB stock solution at 3 $mL \cdot min^{-1}$ into an incoming flow of well water of 1490 $mL \cdot min^{-1}$ for a 90% replacement in just over 1.5 h. The control fish received the TCMTB carrier only, at the same delivery rate. The exposure tanks were visually separated with black curtains and overhead cover was provided to the fish under a 30-cm sector of black plastic. Under these simulated stream conditions, juvenile salmonids maintain position against the current and prefer to stay loosely schooled close to cover, a predictable behaviour which facilitates general observation of any changes (Kruzynski 1972, 1979). General visual observations on swimming activity, relative location in the channel, schooling, and respiration were made throughout the day through slits in the plastic curtains. A wave of the hand across the channel was used as stimulus for fright or startle response by the fish.

## Prey Transfer

After a 48-h "exposure" period, diluent water flow was stopped and 700 mg of MS-222 dissolved in 1 L of seawater was added directly to the annular tank in the vicinity of the intake of the recirculating pump. This ensured rapid mixing and avoided localized concentrations of anaesthetic. Because the annular tanks were designed to facilitate the rapid capture of schooling salmonids, handling stress was minimized.

TABLE 2. Temperatures (°C) of water supplies during acclimation and exposure of chinook salmon to TCMTB in annular tanks.

| | Acclimation | Exposure |
|---|---|---|
| Trial 1 (June 20–27)[a] | | |
| Min. | 9.8 | 9.5 |
| Mean | 10.5 | 9.8 |
| Max. | 11.4 | 10.2 |
| Trial 2 (July 3–10)[b] | | |
| Min. | 9.6 | 9.6 |
| Mean | 10.0 | 10.5 |
| Max. | 10.7 | 11.9 |

[a] On well water June 20 at 16:30 to June 25 at 10:00; on municipal water for the subsequent 48-h exposure period ($n$ = 168).

[b] Both acclimation and exposure on well water ($n$ = 161).

Within 5 min of anaesthetic addition, fish were collected, counted, and placed in a darkened recovery/transport bucket containing brackish water. Control and exposed fish were pooled and transferred to the 15 500-L predation tank where a further 5-min recovery period was provided prior to completely submerging the bucket in the freshwater lens of the deep tank and allowing the prey to swim out.

Other than the occasional careful visual observation from the periphery, the predation tank remained undisturbed for the duration of each predation trial. Five days later, the tank was drained, predators and kelp removed, and the surviving prey killed with MS-222 for enumeration. Fork lengths and wet weights were determined and identity according to fin clip was recorded.

Predators and kelp were replaced and the tank refilled with seawater. After a 2-d recovery period, a 4-d ration of live prey taken from a stock rearing tank was provided to the rockfish which were then starved for 1 wk prior to the next experimental prey addition.

## TCMTB

Stock solutions of TCMTB were made up from a sample of Woodstat 30WB provided by Diachem Industries Ltd., Richmond, B.C. This formulation is listed as a 30% active aqueous dispersion of TCMTB and is synonymous with Busan 30WB; both products are manufactured by Buckman Laboratories, Memphis, Tenn., and shipped to local distributors for sale to antisapstain treatment facilities. A sample of the carrier (RHM 721/184) defined by the manufacturer as "containing all ingredients at the proper concentrations found in Busan 30WB except for TCMTB" was obtained from Buckman Laboratories and served as the carrier control. Although the exact identity of the carrier solvents remains proprietary information, the carrier is known to be a mixture of surfactants and emulsifiers. The industrial formulation rather than active ingredient was used to simulate existing use and discharge patterns in British Columbia. Ancillary experiments (Kruzynski and Birtwell 1991), which measured respiratory responses of juvenile salmon, were conducted with pure TCMTB and yielded results similar to those of Woodstat 30WB whereas the carrier had no effect.

Stock solutions of TCMTB or carrier were made up by dissolving weighed amounts in 100 mL of distilled water (DW) and transferring quantitatively into black-painted glass Mariotte bottles containing 12 L of vacuum-degassed DW.

TABLE 3. Experimental design of predation trials conducted with chinook salmon smolts after a 48-h exposure to 10 $\mu$g TCMTB·L$^{-1}$ June 20 and July 3, 1991.

| | Trial 1 | | | Trial 2 | | |
|---|---|---|---|---|---|---|
| | Control | | Exposed | Control | | Exposed |
| Pelvic clip | Left | | Right | Left | | Right |
| Acclimation time (d) | | 4.3 | | | 4.8 | |
| Mortalities during exposure ($n/n_i$)[a] | 0/48 | | 2/50 | 0/62 | | 2/62[b] |
| Number transferred to predation tank ($n$) | 47[c] | | 47[c] | 59[b] | | 59[b] |
| Predation interval (d) | | 5 | | | 5 | |
| Numbers of salmon consumed | | | | | | |
| ($n$) | 5 | | 38 | 10 | | 44 |
| % | 11 | | 81 | 17 | | 75 |
| Selection index against TCMTB[d] | | 0.77 | | | 0.63 | |
| $\chi^2$ | | 46.7[d] | | | 39.5[d] | |

[a] $n_i$ = number of salmon at start of toxicant exposure.

[b] One additional exposed fish died during transfer to predation tank; control "$n$" was reduced by 1 to equalize numbers.

[c] One additional exposed fish was unaccounted for, so control "$n$" was reduced by 1 to equalize numbers.

[d] Selection index (Dice 1949) highly significant ($P < 0.001$).

After making up to a net weight of 18 kg with DW, the stock bottles were installed in the annular tank exposure system. Diluent water flow and toxicant drip rates were checked three times daily and adjusted as required.

The present study is only one component of a broader investigation of the aquatic toxicology of TCMTB which we began in 1989. Over this period, we have confirmed by HPLC techniques developed by Environment Canada (Krahn 1990) that our exposure system can maintain TCMTB concentrations within ±1 $\mu$g·L$^{-1}$ nominal in the 10 $\mu$g·L$^{-1}$ range. Periodic analyses confirmed no change in the percent active ingredient of TCMTB (27.9%) over the 3-yr interval since October 1988. Thus, although no water samples were analyzed from either predation trial, we are confident that the nominal exposure concentrations of 10 $\mu$g·L$^{-1}$ were achieved.

Midway through the first predation experiment (Trial 1), an unscheduled interruption of our laboratory well water supply necessitated a change to dechlorinated municipal water. This occurred on the last day of the annular tank acclimation period. These two water supplies differ significantly in their chemistry: the dechlorinated municipal water is significantly more acidic and of poorer buffering capacity than well water (Table 1) which we normally use for stock rearing and experimentation. However, by gradual admixing of municipal to well water, we managed to modulate the temperature differences (Table 2) and provided a gradual transition between the two. Because of the temporary uncertainty of supply of well water and to avoid additional complications, we chose to complete Trial 1, including the stratification of the predation tank, with municipal water. Trial 2, on the other hand, was conducted entirely on well water.

## Results

The acute toxicity of TCMTB to the same stock of chinook salmon used in the present study was determined in June 1989 by MacKinnon (1991). Flow-through bioassays conducted under simulated stream flow conditions in our annular tanks yielded a 96-h LC50 of 10.3 $\mu$g·L$^{-1}$ (95% confidence limits 9.2–11.6). The time-to-mortality data indicated that a 48-h exposure to 10 $\mu$g·L$^{-1}$ TCMTB represented the onset of the acute toxicity threshold to the most sensitive fish in the test population. This was confirmed in the present study, with mortalities just beginning towards the end of the exposure period. The acute toxicity of TCMTB was similar in both experiments over the 48-h exposure as indicated by 4 and 3% mortality, respectively (Table 3). In both cases, mortalities occurred overnight during the last 12–15 h of exposure. However sublethal effects, such as increased cough rate, were evident within 2 h of the start of toxicant addition.

### Salmon Behaviour during Exposure

Within 6 h of the start of TCMTB addition, exposed fish could be differentiated from controls by a progressive reduction in the tightness of schooling under the shaded sector of the annular tank. However, in spite of marked changes in schooling behaviour, fright or escape response to hand movement remained indistinguishable from controls after 36 h of exposure. By 48 h, TCMTB-exposed fish were partially scattered around the circumference of the tank, but except for a few more severely affected individuals which had sought refuge in areas of reduced water velocity, fright response was still maintained, albeit at a reduced level.

Respiratory response proved to be the most rapidly and readily observed symptom of TCMTB exposure. Within a day, exposed fish could clearly be distinguished from controls by the presence of continuously flared opercula and the strikingly elevated cough rate characteristic of sublethal TCMTB toxicity (Kruzynski and Birtwell 1991).

### Predation

Both predation experiments indicate that prior TCMTB exposure rendered chinook salmon significantly more vul-

TABLE 4. Lengths and wet weights (means ± SD) of chinook salmon surviving 5-day predation trials.

| | Pelvic fin clipped | Weight (g) | Fork length (mm) | *n* |
|---|---|---|---|---|
| Trial 1 | | | | |
| Control | Left | 6.78 ± 1.02 | 89.6 ± 4.8 | 42 |
| Exposed | Right | 6.17 ± 1.57 | 88.1 ± 5.8 | 9 |
| Trial 2 | | | | |
| Control | Left | 9.09 ± 1.52 | 98.4 ± 5.0 | 49 |
| Exposed | Right | 8.62 ± 1.13 | 98.1 ± 3.7 | 15 |

nerable to predation than unexposed controls (Table 3, $P < 0.001$, 1 df). Pooling the results of both trials, of a total mixed population comprising 212 salmon introduced into the predation tank, 82 exposed fish and 15 control fish were consumed, a ratio of 5.5:1.

The lengths and weights of chinook salmon that survived the predation trials are listed in Table 4. Although the data hint of a possible selection by rockfish for slightly larger prey, none of the size differences between exposed and corresponding control salmon were statistically significant (Student's *t*-test; $P \leq 0.05$).

### Behaviour during Predation Trials

During observations, special care was exercised to avoid casting shadows or causing disturbance that might influence the behaviour of predators or prey. As a result of these precautions, the following account represents the results of limited incidental observations ancillary to the overall purpose of the bioassay which was to link behavioural changes observed during exposure to susceptibility to predation. These observations do, however, represent a composite of behaviour noted during several pilot experiments designed to establish the final protocol and are considered to be representative of behaviour during the two experiments reported.

Under isohaline conditions prior to stratification, rockfish preferred the bottom metre of water, although they did at times inhabit kelp within 1 m of the surface. Under vertically stratified conditions, especially after prey addition, the rockfish were seen cruising (a steady continuous swimming) around the tank and moving slowly up to but not above the halocline. Within the limits of observation, rockfish were never seen to enter the freshwater lens, nor did prey addition trigger immediate feeding. Chinook salmon prey quickly sounded and took cover among the kelp and along the walls of the tank. Several hours later, the majority were loosely aggregated throughout the tank, well below the halocline. Nonetheless, in subsequent days, spontaneous excursions through the halocline into freshwater were not uncommon and in preliminary experiments, this movement could be readily induced by carefully sprinkling a small quantity of food pellets onto the surface. Large numbers of salmon quickly rose from depth into freshwater to feed, returning immediately back into seawater below the halocline. During the 5 d after prey addition, TCMTB-exposed salmon, especially those exhibiting elevated cough rates, could be distinguished from controls by their vertical position in the water column. They tended to occupy freshwater or, if in seawater, remained in close proximity to the halocline. Those that appeared to be most seriously affected were often seen above the halocline maintaining position against the current under cover or along the darkened algal-covered walls of the tank. In general, such fish which initially numbered two or three at any given time appeared reluctant to move away from their stations, with the result that their identity could often be confirmed by pelvic fin clip.

TABLE 5. Size of the yellowtail rockfish used as predators on chinook salmon smolts exposed to TCMTB.

| Fork length (cm) | Wet weight (g)[a] | Age (yr)[b] |
|---|---|---|
| 28 | 420 | 5.4 |
| 33 | 615 | 6.9 |
| 38 | 940 | 9.0 |

[a] Determined on July 15, 1991, at completion of predation Trial 2. Fish were not starved prior to weighing.

[b] Calculated from von Bertalanffy equation utilizing data from Archibald et al. (1981).

Salmon generally avoided cruising rockfish; however, at other times, they swam within several body lengths of stationary or slowly moving rockfish which had moved up from depth to a midwater position or were concealed in kelp within 30 cm of the halocline. Attacks by rockfish on chinook salmon were not witnessed. However, since observation periods were limited, rockfish could have detected the presence of the observer or chose to feed at depth or during twilight hours when reduced light intensity made observation impossible.

The predation trials reported in this study were conducted midway through the 6-mo period during which the rockfish were held in captivity at WVL. At the time of release in the last week of September 1991, the rockfish were in excellent condition, showing no sign of abrasion or other usual indicators of the stress of confinement. Table 5 indicates the size and estimated age of the three rockfish predators at the conclusion of predation Trial 2.

## Discussion

Differences in water chemistry during the TCMTB exposure did not appear to have a bearing on the overall results of the subsequent predation trials. In preliminary work, we found that juvenile coho salmon survived a 1-wk exposure to 5 $\mu g$ TCMTB$\cdot L^{-1}$ in freshwater with no mortality. However, when the toxicant was discontinued and the water supply switched from fresh to seawater, 90% of the fish died within 37 h. Because no control fish died, it was clear that prior TCMTB exposure had compromised seawater tolerance in coho salmon (Department of Fisheries and Oceans, unpublished information).

In the present experiments, as in nature, chinook salmon were free to occupy the deeper seawater layer of this simulated estuary and thus behaviourally determine the amount of time spent in fresh, brackish, or seawater. However, the seawater environment contained the predator. This situation likely presented the TCMTB-exposed fish with conflicting choices. The natural tendency to move into deeper water to seek protection offered by the school and shelter provided by kelp and reduced light intensity carried with it the physiological stress of salinity challenge coupled with the psychological stress of predation (Rehnberg and Schreck 1987). Jarvi (1989) reported that the presence of satiated and therefore nonfeeding predators (*Gadus morhua*) elicited a synergistic stress response which resulted in 90% mortality of freshwater-adapted Atlantic salmon smolts during a subsequent routine seawater challenge test. Freshwater-adapted salmon that had not been exposed to predators survived direct seawater transfer with no mortality. Even when smolts were preadapted to seawater, a mortality of 43% occurred after exposure to nonfeeding predators. Seawater-adapted controls not exposed to predators suffered no mortalities.

Hedtke and Norris (1980) reported that the added stress caused by the presence of a trout predator rendered a nonacutely toxic level of ammonia lethal to juvenile chinook salmon. In our case, chinook salmon were not forced to move into seawater and presumably could have behaviourally minimized salinity stress. In the period immediately after prey addition, the three rockfish appeared to increase their swimming speed around the circumference of the tank, suggesting an awareness of the presence of the salmon. However, they did remain within about a metre of the bottom giving no indication of immediate attack, even though salmon were active in their vicinity. By virtue of their entry into rockfish habitat, the predator-naive salmon would likely have been subjected to a combination of stresses from the presence of predators to those elicited during attack.

In his review paper on the significance of sublethal effects, Sprague (1971) underlined the importance of establishing whether measured changes were ecologically meaningful. However, in spite of great strides that have been made in developing physiological and biochemical tests that measure stress in fish, interpretation of their significance remains elusive. Recently, there has been renewed interest in linking stress biomarkers (Hugget et al. 1992) to whole-organism effects. For example, the increased susceptibility to predation of hatchery-reared coho salmon that had undergone routine handling stress was reported by Olla and Davis (1989). The correlation of plasma cortisol dynamics with successful predator avoidance under laboratory conditions has recently been addressed (Olla et al. 1992).

The mixed success of an increasing reliance on hatchery production to maintain Pacific Northwest salmon stocks (Hilborn 1992) has focused attention on losses due to predation after release into freshwater (Patten 1971), during entry into seawater, and during early estuarine residence. Predation on juvenile salmon is now recognized as an important factor limiting survival to adulthood, with the period of transition to estuarine and marine waters representing an especially critical stage during which opportunistic predators can inflict high losses.

The congregations of piscivorous predators that take advantage of hatchery releases of Atlantic salmon have been well documented by Hvidsten and Mokkelgjerd (1987) and Hvidsten and Lund (1988) in Norway and by Larsson (1985) in Sweden. These authors concluded that from 25 to 50% of released smolts were lost to aquatic predators during migration and early estuarine transition. In a reinterpretation of data originally reported by Piggins (1958), Thurow (1966) concluded that predators captured from 50 to 70% of Atlantic salmon parr and smolts in the period following hatchery releases in Ireland. In British Columbia, Mace (1983) found that from 10 to 32% of hatchery-released chinook and coho smolts were taken by piscivorous birds in the estuary of the Big Qualicum River on Vancouver Island. More recently (summer 1992), warmwater temperatures attributable to El Niño resulted in an anomalous intrusion of the normal distribution range of mackerels (*Scomber japonicus* and *Trachurus symmetricus*) into Barkley Sound and Alberni Inlet, Vancouver Island. Examination of stomach contents of mackerels caught in very large numbers in purse seines suggested that most of the 10 million chinook salmon smolts released from the federal government Robertson Creek Hatchery had fallen prey to these normally pelagic predators (Department of Fisheries and Oceans, unpublished information).

One would thus intuitively expect that the cumulative effects of natural stresses involved in the transition to seawater (Jarvi 1990) could be potentiated by further debilitation or reduction in "ecological fitness" brought on by exposure to xenobiotics or adverse environmental conditions in the wild. In fact, Basch and Truchan (1976) observed increased predation by gulls on disorientated fish that had encountered chlorinated power plant effluents. We have observed comparable concerted aerial attacks on a variety of fish species floundering near the surface as a result of hypoxic conditions in the Fraser River estuary (Birtwell et al. 1983).

One would expect that a corresponding increase in predation by opportunistic piscivorous fish must also occur, although we are unaware of documented cases relating sublethal xenobiotic exposure to increased predation by fish. However, McLane (1950) reported that the addition of rotenone (derris) to a Florida pond elicited a change in the behaviour of a normally quiescent bottom-dwelling fish, the Florida swamp darter (*Hololepis barrati*), which rendered it approximately eight times more susceptible to predation by largemouth bass (*Micropterus salmoides floridanus*).

The behavioural differences between control and TCMTB-exposed chinook salmon confirm the results of ancillary experiments which have demonstrated a general reduction in swimming speed, as measured by spontaneous exploration activity and speed of escape to shelter (Chew et al. 1991; Kruzynski et al. 1991). In addition to such effects which can be considered indicators of volitional swimming, sublethal TCMTB exposure was shown to reduce swimming stamina (against an imposed current) of chinook and coho salmon and rainbow trout (MacKinnon 1991). However, MacKinnon determined critical swimming speed by stepwise increments of velocity ranging from 15 to 30 min in duration, which represents sustainable or cruising speed; thus, it is unlikely that this measure would reflect a decrease in the ability to evade predator attack. A combination of increased response latency and perhaps a reduction in burst speed (Brown et al. 1985) which would govern repeated escape attempts is more likely to have contributed to the observed increase in vulnerability of chinook salmon. MacKinnon's (1991) findings of a significant increase in plasma lactate in juvenile coho salmon following a 48-h exposure to 10 $\mu g$ TCMTB$\cdot L^{-1}$ lend credence to this speculation.

The gradual disruption of schooling in chinook salmon exposed to TCMTB corroborates the sensitivity of this behavioural response to toxic stress. The annular exposure tanks we used to expose salmon prey prior to the predation trials were designed to take advantage of the natural tendency of salmonids to aggregate under cover and maintain position against a current. A progressive increase in fish-to-fish distance was observed in brook trout (*Salvelinus fontinalis*) sublethally exposed to Methoxychlor through the diet (Kruzynski 1972) and in sockeye salmon (*Oncorhynchus nerka*) during sublethal aqueous exposure to dehydroabietic acid (Kruzynski 1979); both of these studies used the annular tank exposure system. Similar disruptions in the schooling behaviour of juvenile chinook, chum (*Oncorhynchus keta*), and coho salmon have also been recorded in waters receiving acutely toxic pulp mill effluent (Birtwell and Kruzynski 1989). Drummond et al. (1986) reported loss of schooling in fathead minnow (*Pimephales promelas*) to be the most sensitive indicator of stress in acute flow-through bioassays for 133 of the 139 organic chemicals tested. In the present study, schooling disruption persisted for several days after transfer of the chinook salmon to the predation tank, suggesting a long-lasting effect that persisted well into the period of recovery from prior toxicant exposure. Since schooling is a well-known antipredation strategy (Cushing and Harden-Jones 1968), it is likely that even subtle aberrations of normal schooling tendency could render toxicant-exposed fish less able to evade predators. Subtle changes in schooling behaviour of fathead minnow as a result of cadmium exposure resulted in increased risk of predation by largemouth bass (Sullivan et al. 1978).

Our results corroborate the utility of Goodyear's (1972) predation bioassay as a tool for establishing the ecological relevance of commonly measured biomarkers of sublethal stress or those used as indicators of xenobiotic exposure. As a whole-organism response, not only can the predation bioassay integrate the effects or interactions of mixtures of toxicants or complex effluents, it can be a powerful experimental tool for investigating the modifying effects of naturally occurring physicochemical factors such as salinity, temperature, and dissolved oxygen. Depending on the ingenuity of the experimental design, such stresses could be imposed during fixed exposure periods, during pulse doses, and/or during subsequent recovery.

Routine laboratory studies on the toxicity of xenobiotics rarely take into account the fate of organisms after conclusion of the exposure period. Conventional protocols developed to determine maximum acceptable toxicant concentration (MATC) values generally disregard additional stresses that an organism is likely to encounter in the wild, in spite of the cumulative nature of the physiological stress response (Barton et al. 1986). Consequently, other than the determination of chemical residues of persistent xenobiotics, the legacy or long-term consequences of prior sublethal exposure have received limited attention in the aquatic toxicological literature.

Wright (1976) emphasized the importance of considering the "recovery period" in interpreting the median effective concentration (EC-50) data as measured by "knockdown" concentrations of oil dispersants to barnacle nauplii. Wright argued that the prediction of biological effects based on EC-50 results would lead to an overestimation of environmental impact on organisms which might be temporarily debilitated but which subsequently have the opportunity to recover between fluctuating or pulse exposures.

In contrast, Lanno (1990) and Lanno and Dixon (1991) reported that rainbow trout exposed to sublethal concentrations of thiocyanate over a period of several months subsequently died of "sudden death syndrome" (SDS) induced by the stress of routine weighing procedures. These observations provide a clear example of the thus far poorly understood interactions of the cumulative effects of xenobiotic exposure and subsequent stressors. Our study demonstrated that prior sublethal exposure to TCMTB led to the death of salmon from predation, results which raise the fundamental question of whether "ecological death" might in many cases in fact be an overlooked legacy of physiologically sublethal toxicant exposure.

An operational prerequisite of the predation bioassay is the determination of the ET-50, the duration of predation interval that results in the consumption of 50% of the population of prey admitted to the tank. This can be readily determined empirically from biomass consumption over time during the predator rearing period. Once set up, the predation bioassay is essentially self-tending, requiring a prey preexposure followed by a simple enumeration of organisms surviving the predation trial. The experimental duration of predator–prey interaction will depend on the handling stress dynamics of the species being studied as well as site-specific requirements. In our case, a 5-d interval was chosen to ensure the recovery of salmon from minor handling stress, to provide time for them to become familiar with new spatially segregated habitats which included opportunities for cover, and to provide the school with time to develop predator avoidance behaviour. In addition, our experimental design incorporates a novel approach to salinity challenge. Rather than the usual instantaneous (forced) transfer from fresh to seawater, we provide a volitional aspect which we feel more closely simulates the natural situation, where salmon are free to choose the proportion of time they spend in fresh, brackish, or seawater. Should a given fish choose to enter seawater immediately and remain there, extensive experimental data in the literature indicate that most major physiologically adaptive hydromineral adjustments have been completed within 5 d. Although our experimental protocols were tailored to chinook salmon subjected to xenobiotic exposure under specific conditions, we suggest a broader potential utility of this approach to other species and situations. For example, a predation interval of hours or minutes would be more appropriate in situations where debilitation is relatively short term such as a result of thermal shock (Sylvester 1972; Coutant 1973) or where predators are known to congregate to take advantage of prey that are disoriented after passage through dams (Beamesderfer and Rieman 1991; Vigg et al. 1991) or debilitated after encounters with chlorinated power plant effluents (Basch and Truchan 1976). In the case of such very short-term acute effects, subtle behavioural changes are rendered relatively unimportant and numerous replicates with fewer fish per trial would comprise a more appropriate experimental design.

From a toxicological perspective, a potential complication could be the gradual translocation of persistent contaminants to the predators, which could in turn affect their appetite or feeding behaviour. This could be avoided by a more frequent replacement of predators. On the other hand, the predation bioassay could conceivably be used in food-chain contaminant transfer studies incorporating xenobiotic-exposed prey organisms. In our case, a protracted predator exposure interval was selected to simulate the recovery from toxicant exposure, which in the case of the discharge of antisapstain chemicals is governed by rainfall and therefore periodic. Additionally, salmon have the opportunity to move in and out of effluent plumes, thereby behaviourally determining exposure and recovery intervals. By providing the opportunity for recovery, our simple "temporal black-box" design would tend to measure the longer term, more persistent consequences of sublethal toxicant exposure. Although analytical techniques to quantify uptake and distribution of TCMTB and its major metabolite (2-mercaptobenzothiazole) in tissue were not available at the time of our study, physicochemical characteristics suggest that significant bioconcentration is unlikely (Brownlee et al. 1992). This suggests that "ecological death" can also occur as a latent consequence of short-term exposure to xenobiotics which do not necessarily accumulate or are quickly depurated.

In an investigation of predation of cutthroat trout (*Oncorhynchus clarki*) on sockeye fry, Bams (1967) discussed the concept of "passive selection" whereby some prey can behave in a manner that renders them more susceptible to attack. In the present study, it was highly probable that the TCMTB-exposed salmon fell into this category. Bams also made the important point that the selective cropping of susceptible prey would rapidly reduce their availability relative to the unaffected fish. Thus, it follows that our results could actually underestimate the relative susceptibility to predation of TCMTB-exposed fish. Herting and Witt (1967) demonstrated experimentally that four species of fish which, as a result of poor condition due to handling stress or disease, were slower moving and did not exhibit normal evasive responses were consistently more susceptible to predation by bowfin (*Amia calva*) than controls. That vulnerability depends on prey behaviour has been demonstrated in the field downstream of dams on the Columbia River, where selective predation by gulls (Ruggerone 1986) and fishes (Beamesderfer and Rieman 1991; Vigg et al. 1991) on temporarily disoriented and therefore more vulnerable juvenile salmonids has led to severe losses.

Under our laboratory conditions, the survival of chinook salmon smolts was clearly jeopardized by sublethal exposure to TCMTB prior to the transition to seawater. Because predator avoidance represents successful integration of biochemical, physiological, and behavioural responses, we consider the predation bioassay to be a valuable addition to current attempts at understanding the significance of the multitude of complex multifactorial interactions that determine environmental risk. In simplistic terms, if survival is to eat and not be eaten (Olla and Davis 1988), then the predation bioassay provides an ecologically relevant means of quantifying survival.

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# Consequences of Pulsed DC Electrofishing and Air Exposure to Rainbow Trout (*Oncorhynchus mykiss*)

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Electrofishing, a technique widely used for fish collection, is a procedure that is often followed by handling and air exposure before fish are released. In this study the pathological and physiological consequences of capture with pulsed DC (pDC) electroshock with and without the additional disturbance of air exposure were examined on rainbow trout (*Oncorhynchus mykiss*). Electroshocked fish showed no evidence of direct mortality, and skeletal damage was only induced under the most severe conditions (~600-g fish, 600 V, 20 or 40 s). Physiological disturbances induced by pDC, in the absence of physical damage, consisted of lactacidosis and stimulation of the interrenal stress response. The acidosis was likely induced by tetany during immobilization and apnea during immobilization and early recovery. In fact, the physiological disturbances induced by pDC were similar in magnitude and duration to 2–3 min of exhaustive exercise and generally more severe than responses previously reported for nonpulsed (i.e., continous) DC gear. In addition, following pDC, fish appeared to experience a mild narcosis, as was evident by a reduced response to the additional stress of air exposure.

La pêche à l'électricité, une technique largement utilisée pour capturer le poisson, est souvent suivie d'une manipulation et d'une exposition à l'air avant la remise à l'eau. Dans cette étude, les conséquences pathologiques et physiologiques de la capture au moyen d'un choc électrique de courant continu pulsé, avec ou sans stress additionnel causé par l'exposition à l'air, ont été étudiées chez la truite arc-en-ciel (*Oncorhynchus mykiss*). On n'a observé aucun signe de mortalité directe chez les poissons ayant subi un choc électrique, et seules les conditions les plus sévères (poissons de ~600 g, 600 V, 20 ou 40 s) ont provoqué des dommages squelettiques. Les troubles physiologiques provoqués par cette méthode, en l'absence de dommages physiques, consistaient en une lactacidose et la stimulation de la réponse interrénale au stress. L'acidose était probablement provoquée par la tétanie durant l'immobilisation et l'apnée durant l'immobilisation et les premiers signes de récupération. En fait, les troubles physiologiques provoqués par le courant continu pulsé étaient assimilables en ampleur et en durée à un exercice exténuant pendant 2 à 3 min et étaient en général plus graves que ceux provoqués par les appareils qui produisent un courant continu non pulsé. En outre, les appareils produisant un courant continu pulsé semblaient provoquer une légère narcose chez les poissons, comme en témoigne une diminution de la réponse à un stress additionnel causé par l'exposition à l'air.

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Electrofishing is used extensively to collect fish for monitoring and fisheries research. The goal of much of this work is to release fish unharmed back to their natural environment following such treatments as measuring for length and weight, scale sampling, tagging, and/or collecting eggs and milt. During processing, fish are often exposed to air for extensive periods. Since this work is often conducted on small and sensitive populations, it is vital to establish what damage might be caused to the fish. A number of authors have reported direct mortality (Hauck 1949; Pratt 1954; Horak and Klein 1967; Hudy 1985; Barrett and Grossman 1988) and spinal injury (Hauck 1949; McCrimmon and Bidgood 1965; Hudy 1985; Sharber and Carothers 1990) as a result of AC and DC electrofishing. Physiological studies conducted on fish exposed to continuous DC electroshock have reported elevated levels of the stress hormone cortisol (Schreck et al. 1976; Woodward and Strange 1987; Mesa and Schreck 1989) and impaired ventilation during and immediately following treatment (Schreck et al. 1976). In contrast, the effects of pulsed DC (pDC) are not well known despite the widespread use of pDC electrofishing gear and the evidence that it is more detrimental to fish than continuous DC (Lamarque 1990). In addition, no studies, to our knowledge, have considered the combined effects of pDC and subsequent air exposure and handling.

This study assessed the potential physical damage, mortality, and physiological disturbances experienced by rainbow trout (*Oncorhynchus mykiss*) as a result of capture with a pDC backpack electrofisher followed by handling. To simulate handling during field processing, fish were exposed to air for various times following electroshock treatment. Physical damage was assessed on the basis of spinal X rays, and survival was monitored following various electroshock and air exposure regimes. The assessment of physiological disturbances tested the hypothesis, based on studies of continuous DC electrofishing, that the responses of fish can be attributed to a combined effect of stimulation of the stress response and impaired ventilation (Schreck et al. 1976; Mesa and Schreck 1989). Due to the mode of immobilization with pDC electrofishing (tetany with pDC versus galvanonarcosis or electroanaesthesia with continuous DC; Lamarque 1990), physiological parameters associated with muscle exertion were also examined.

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Stress levels were assessed by measurements of plasma cortisol, glucose, and catecholamines. The first two measurements are widely used as stress indicators and have been examined in a number of studies of continuous DC electroshock (Schreck et al. 1976; Woodward and Strange 1987; Mesa and Schreck 1989). Catecholamines, in contrast, are rarely examined, but are also significantly elevated by stress (Mazeaud et al. 1977). As in previous electroshock studies, blood lactate was examined as a measure of anaerobic metabolism. In addition, the consequences of pDC to acid–base balance were examined by measurements of blood pH and total $CO_2$, since such disturbances typically follow any severe muscular exertion (Wood 1991).

## Materials and Methods

### Experimental Animals

Rainbow trout (30–600 g) were obtained from Rainbow Springs Trout Farm in Thamesford, Ont. Fish were held for at least 2 wk before experimentation in well-aerated, 400-L circular tanks supplied continuously with dechlorinated municipal tap water. Fish were fed three times weekly with commercial trout pellets (Martins Feed Mills, Elmira, Ont.).

### Electrofishing Procedure

All experiments were conducted in a rectangular (2.4 × 0.6 m) test tank; fish were transferred to a central screened section of the tank 0.3 m long with the cathode of the electrofisher fixed at 0.3 m from the screen at one end and the anode 1.2 m from the opposite screen. Within the central section, fish were either permitted to swim freely or, for those fitted with blood-sampling catheters, confined in 2-L darkened acrylic boxes (6.5 × 7.0 × 45 cm, W × D × L). The temperature ($T$) and conductivity varied between 8 and 20°C and 240 and 270 $\mu$mhos·cm$^{-1}$, respectively. Electrofishing was conducted with a Smith–Root VII pDC backpack electrofisher set at 200–600 V for a 20- to 40-s duration, with the pulse duration and frequency settings at 5 ms and 60 Hz, respectively. Such pulse duration and frequency settings are commonly used to capture trout in hard waters typical of southern Ontario (R. Dalziel, Ontario Ministry of Natural Resources, 90 Shepard Ave. E., 5th Floor, North York, ON M2N 3A1, personal communication).

### Experimental Protocols

*Spinal damage assessment*

Rainbow trout (at $T = 7 \pm 1$°C) of three size classes (~50, ~100, and ~600 g) were electroshocked at a constant distance from the electrodes as specified above. Four different electroshock treatments were used (200 V for 20 s, 400 V for 20 s, 600 V for 20 s, or 600 V for 40 s). Immediately following electroshock, fish were terminally anaesthesized in tricaine methanesulfonate (MS222; 2 g/L; Syndel Laboratories Inc., Vancouver, B.C.) and then frozen for later examination. Both lateral- and dorsal-view X rays were taken on Kodak Industrex M film with a Hewlett Packard Faxitron 43805N X-ray system.

*Survival*

Survival following electroshock and air exposure was monitored in two trials. In the first trial, fish ($N = 10$ per group, four groups, weight = 80–120 g) were fin-clipped under water for identification and then acclimated to the test tank for 3 d prior to experimentation. Fish were not fed during this time. Electrofishing was conducted using the above procedure. Immediately following electroshock, fish were netted into a container lined with moistened paper towels, where they were exposed to air for 0, 1, 2, or 4 min and then transferred to a partially covered circular 400-L holding tank where survival was monitored for 2 wk. Temperature during treatment and recovery ranged between 15 and 20°C. In the second trial, non-fin-clipped trout ($N = 20$ per group, four groups, weight = 30–50 g) were treated as in the first trial. Following treatment, each group was kept separate in partially covered, 50-L tanks at 13 ± 1°C, where survival was monitored.

*Behaviour*

Response time and duration of apnea (i.e., absence of ventilation) were quantified in a separate trial. Free-swimming rainbow trout and trout confined to boxes ($N = 22$, weight = 125.7 ± 7.5 g, $T = 16$°C) were electroshocked for 20 s, using the standard procedure (see above). Within this period, the time required for fish to turn over was recorded and defined as response time. Following electroshock, the duration of apnea was also recorded.

*Physiological studies*

Two blood sampling techniques, caudal severance and surgically implanted dorsal aorta (DA) catheters, were employed in different trials. Sampling by DA catheters was necessary for measurements of catecholamine levels and acid–base status, as these measurements are highly sensitive to sampling stress, but the chronic stress associated with anaesthetization and surgery (Heisler 1984; McDonald and Milligan 1992) may be problematic. Consequently, blood was also collected by caudal severance, which eliminates the chronic stress. The physiological response to electroshock combined with air exposure was examined using the DA cannulation technique only. Temperature for all physiological studies was 16 ± 1°C.

*Electroshock: blood sampled by caudal severance* — Rainbow trout ($N = 70$, weight = 113.3 ± 3.6 g) were acclimated to the test tank 24 h prior to treatment and were not fed during this time. Free-swimming fish were electroshocked, as specified above, and killed with a blow to the head. Blood was collected by severing the tail about 1 cm posterior to the anus ($t$ + 5 min at 0, 1, 2, 4, 8, and 24 h). The blood was collected within 30 s into heparinized collection tubes. Subsamples of whole blood (50-$\mu$L aliquots) were fixed in 200 $\mu$L of ice-cold 8% perchloric acid (PCA), centrifuged for 5 min, and the supernatant stored at 4°C for later analysis of lactate. The remaining blood was centrifuged for 5 min and the plasma drawn off and frozen for subsequent measurements of cortisol and glucose.

*Electroshock: blood sampled by DA catheter* — Rainbow trout ($N = 7$, weight = 355.3 ± 20.7 g) were fitted with catheters in the dorsal aorta under MS222 anaesthesia (0.8 g/10 L). Fish were allowed at least 48 h to recover, unfed, from the surgery and anaesthesia in 2-L darkened

TABLE 1. Number of fish showing spinal damage (numerator) in three size groups of fish ($N$ = denominator) exposed to pDC electroshock at various voltages for 20 or 40 s durations (a dash indicates condition not tested.

| Fish size (g) | Electrofishing treatment 200 V 20 s | 400 V 20 s | 600 V 20 s | 600 V 40 s |
|---|---|---|---|---|
| 47.2 ± 2.7 | 0/5 | — | 0/20 | — |
| 103.4 ± 6.2 | 0/5 | 0/5 | 0/10 | — |
| 593.6 ± 23.4 | 0/5 | — | 1/10 | 1/5 |

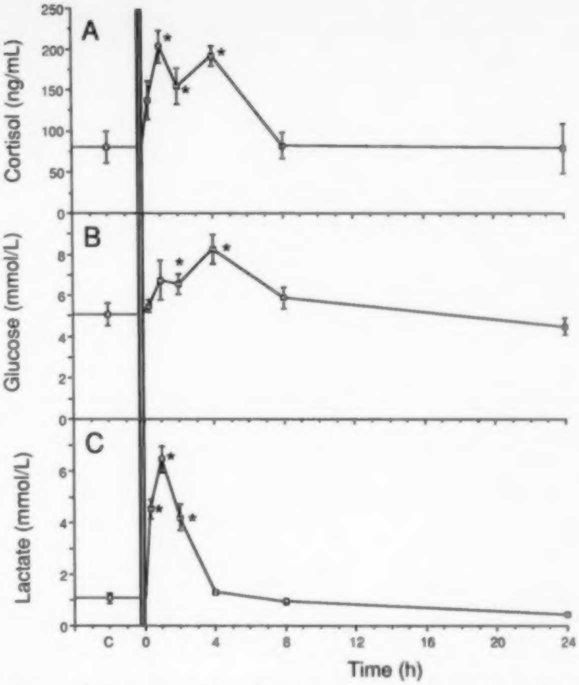

FIG. 1. Influence of 20-s electroshock on plasma (A) cortisol, (B) glucose, and (C) whole-blood lactate sampled by caudal severance (shaded bar indicates the electroshock treatment). All values are expressed as mean ± 1 SEM ($N = 7–10$); $T = 17°C$; *significant difference from resting ($p < 0.05$).

acrylic boxes before being treated. One day prior to treatment, fish were moved into the test tank, still confined in the boxes, but with a constant exchange of water (5 L/min) between the boxes and test tank. Electroshock was applied as above to fish held in boxes. Blood collected from the catheter (at 0, 1, 2, 4, 8, and 24 h) was immediately measured for pH, total $CO_2$, and hemoglobin. Subsamples of blood were processed for lactate (as above), and the remaining blood (~0.3 mL) was centrifuged for 5 min and plasma drawn off and frozen for subsequent measurements of catecholamines, cortisol, and glucose.

*Electroshock and air exposure: blood sampled by DA catheter* — The protocol for this experiment was similar to that above, except that immediately following electroshock the boxes were lifted out of the tank and inverted to drain (<10 s). The fish were then exposed to air for 1 min before being returned to the test tank.

### Analytical Procedures

Arterial pH was determined on 50-μL samples injected into a Radiometer pH microelectrode (type G297/G7) attached to a Radiometer PHM 71 Mk 2 acid–base analyzer. Total $CO_2$ was measured on 50 μL of arterial blood in a Corning (model 965) $CO_2$ analyzer. Arterial $CO_2$ tension ($P_{CO_2}$) and the metabolic acid load ($\Delta H^+_m$) were then determined. $P_{CO_2}$ was calculated using experimentally determined pK′ and $CO_2$ solubility coefficients for trout blood (Boutilier et al. 1984). $\Delta H^+_m$ was calculated over each time period (1–2) by the equation of McDonald et al. (1980):

$$\Delta H^+_m = [HCO_3^-]1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2)$$

where β, the nonbicarbonate buffering capacity of the blood, was estimated using the following formula (Wood et al. 1982):

$$\beta = 1.1 \times [\text{hemoglobin}] - 2.5.$$

Whole-blood lactate was determined on 100-μL aliquots of PCA extract, using the lactate dehydrogenase method, with Sigma reagents. For determination of hemoglobin concentrations, 20 μL of whole blood was diluted in 5 mL of Drabkins solution and read against hemoglobin standards on a Phillips PYE Unicam PU 8600 UV/VIS spectrophotometer. Catecholamines were extracted from 100-μL plasma aliquots by binding to alumina in a Tris–EDTA buffer at a pH of 8.6. The pellet was washed twice with double-distilled water and then resuspended in 0.1 mol PCA/L. A 70-μL aliquot of extract was separated by high-performance liquid chromatography (HPLC; Waters model 510) with a Waters M460 electrochemical detector. Individual catecholamines were identified and quantified by comparisons with extracted and unextracted catecholamine standards. Cortisol was measured on 20-μL aliquots of plasma using a radioimmunoassay kit (Kallestead Canada Inc. Quanticoat™ Cortisol RIA Kit 825) validated for use on trout plasma (Hontela et al. 1992) that involves the competitive binding of plasma cortisol and $^{125}I$-labelled cortisol to cortisol-specific antibody coated tubes. Samples were counted on a gamma counter (Minaxi Auto-gamma® 5000 series). Plasma glucose concentrations were measured on 10-μL aliquots using the hexokinase enzymatic assay (Lowry and Passonneau 1972) with Sigma reagents.

### Statistical Analysis

All values are expressed as mean ± 1 standard error of the mean (SEM). Significance was determined by Student's $t$-test at $p < 0.05$. Data obtained from DA catheterization trials were compared using the paired $t$-test whereas all other comparisons were made via the unpaired $t$-test.

## Results

### Physical Damage and Mortality

Trout electroshocked for 20 s at 200 V and then air exposed for either 0, 1, 2, or 4 min did not exhibit any mortality in the 2 wk period following treatment.

X-ray analysis of 65 fish revealed three conditions: no damage; naturally occurring, fused abnormalities; and electroshock-induced spinal damage. The latter was identifiable by the appearance of misaligned vertebrae. Such

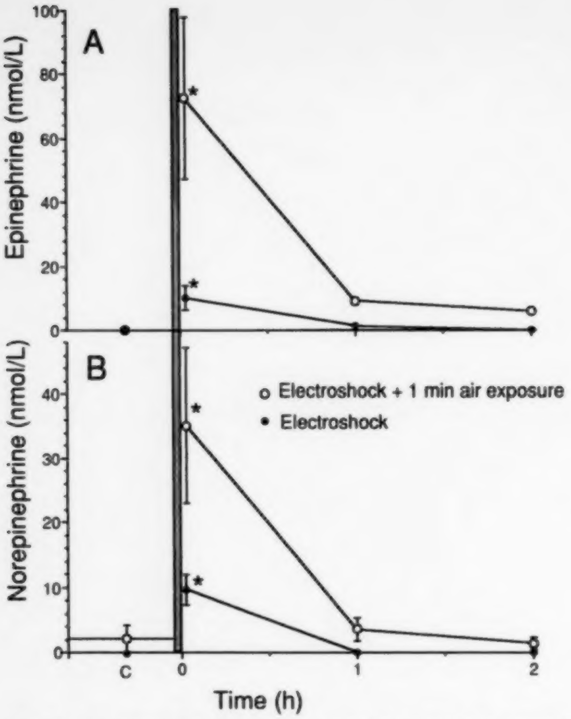

FIG. 2. Changes in plasma (A) epinephrine and (B) norepinephrine in catheterized electroshocked fish following a 20-s electroshock (solid circles) and a 20-s electroshock followed by 1 min of air exposure (open circles) (shaded bar indicates the treatment period). All values are expressed as mean ± 1 SEM ($N = 4-7$); $T = 16 \pm 1°C$; *significant difference from resting ($p < 0.05$).

injuries were only observed in two large trout (~600 g) treated with 600 V for either 20 or 40 s (Table 1). Injuries in both fish involved three vertebrae. No injuries were observed in fish of smaller size or treated with lower electroshock voltages.

### Behaviour

Free-swimming trout responded to the pDC current in 1.9 ± 0.3 s ($N = 15$). Response of fish confined in acrylic boxes was harder to observe, but by 2.9 ± 0.3 s ($N = 7$), all had clearly responded. Each group was in tetany for the remainder of the 20-s electroshock. Immediately following electroshock, ventilation was absent in free-swimming fish for up to 3 min, with an average duration of 19 ± 13 s. Ventilation could not be accurately observed for fish confined to boxes.

### Physiological Effects

Electroshock elevated plasma cortisol about twofold above resting levels (81.0 ± 19.0 ng/mL), with the peak occurring at 1 h after treatment, and resting levels did not return until 8 h after treatment (Fig. 1A). An even more rapid and substantial elevation in catecholamines occurred, from below detection limits (ie., < 2–3 nmol/L) under resting conditions to peaks of about 10 nmol/L for both plasma epinephrine and norepinephrine immediately following electroshock. Catecholamine levels had fully recovered by 1 h into the recovery period (Fig. 2A and 2B). Plasma glucose levels rose more slowly than either cortisol or catecholamines from a resting value of 5.1 ± 0.5 mmol/L to a peak of 8.2 ± 0.5 mmol/L at 4 h postelectroshock with recovery by 8 h (Fig. 1B).

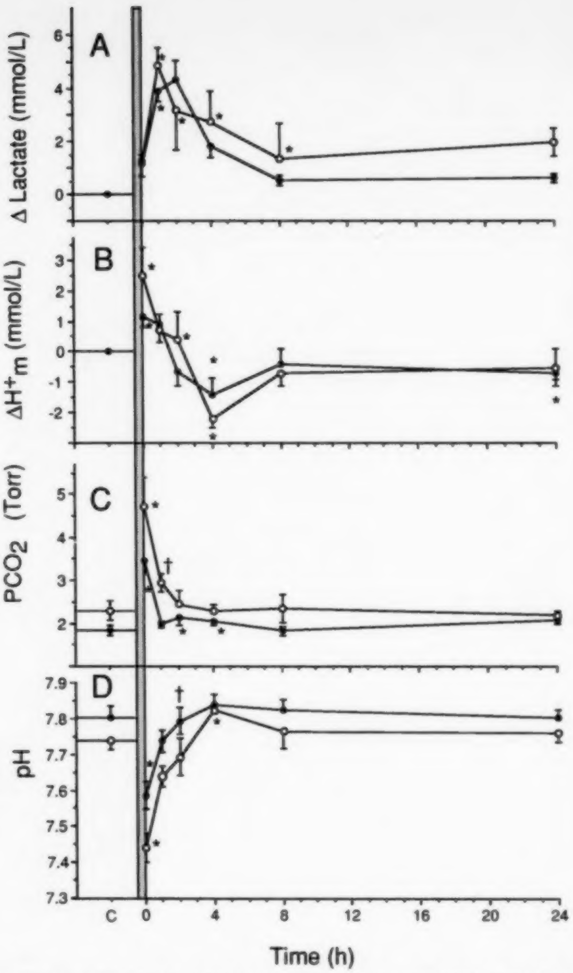

FIG. 3. Changes in blood (A) lactate, (B) metabolic acid load ($\Delta H^+_m$) (C) $P_{CO_2}$, and (D) pH in catheterized fish following a 20-sec electroshock (solid circles) and a 20-s electroshock followed by 1 min of air exposure (open circles) (shaded bar indicates the electroshock and air exposure treatment). All values are expressed as mean ± 1 SEM ($N = 4-7$); $T = 16 \pm 1°C$; *significant difference from resting ($p < 0.05$); †significant difference between treatments.

Electroshock elicited an immediate lactacidosis in the fish. Blood lactate was elevated about sixfold above resting levels (1.1 meq/L) and remained elevated for 2 h (Fig. 1C and 3A). The proton efflux to the blood from the muscle resulted in an immediate metabolic acid load ($\Delta H^+_m$) of 1.1 meq/L (Fig. 3B), the difference between the $\Delta H^+_m$ and the Δlactate (1.1 versus 4 meq/L) (Fig. 3A and 3B) reflecting retention of protons in the muscle. Fish concurrently experienced an extracellular respiratory acidosis as was apparent from the elevation in $P_{CO_2}$ from 1.8 to 3.5 Torr

TABLE 2. Comparison of maximum disturbances (elevation above controls) of cortisol, glucose, and lactate following pDC in the present study and continuous DC (DC) previous studies using salmonids (NR = not reported).

| | Present study | Schreck et al. 1976 | Mesa and Schreck 1989 | Woodward and Strange 1987 |
|---|---|---|---|---|
| Current type | pDC | DC | DC | DC |
| Voltage (V) | 200 | 230 | 300 | 110 |
| Electroshock duration (s) | 20 | 40 | 4 | <5 |
| Water conductivity (μmho/cm) | 240–270 | 227 | NR | 45–50 |
| Water temperature (°C) | 16 | 13 | 12–13 | 15–16 |
| Trout species | Rainbow | Rainbow | Cutthroat | Rainbow |
| Size | 113 g | 169 g | 63 g | 160–211 mm |
| Cortisol (ng/mL) | +130 | +47 | +90 | +40 |
| Glucose (mmol/L) | +3.5 | +2.2 | NR | +1.1 |
| Lactate (mmol/L) | +5.5 | +0.9 | +1.7 | NR |

(1 Torr = 133.322 Pa) (Fig. 3C). These two factors contributed to the 0.22 unit reduction in arterial pH from an initial pH of 7.80; pH recovered by 2 h with an overshoot of 0.04 pH unit at 4 h (Fig. 3D).

*Electroshock and air exposure*

The additional stress of 1 min of exposure to air immediately following electroshock tended to increase the degree of disturbance experienced by the fish. Peak norepinephrine and epinephrine values were 35 and 70 nmol/L, respectively, compared with 10 nmol/L for both catecholamines in fish that were electroshocked only (Fig. 2A and 2B). Disturbances in pH, $\Delta H^+_m$, $P_{CO_2}$, and lactate also tended to be larger in fish subjected to 1 min of exposure to air following electroshock (Fig. 3A–3D). However, none of these elevations was significantly higher than those produced by electroshock alone. In addition, the magnitude and pattern of cortisol and glucose elevations were similar between the two treatments (data not shown).

## Discussion

### Physical Damage

Previous studies have indicated that electrofishing can lead to mortality in at least two ways: rapid mortalities resulting from failure to recover from irregular ventilation patterns (Lamarque 1990) and delayed mortalities resulting from spinal damage and hemorrhaging incurred during violent muscle contractions (Hauck 1949; Schreck et al. 1976; Hudy 1985; Lamarque 1990). Of the three types of electrofishing currents (AC, continuous DC, and pDC), AC is thought to be the most severe, as it is known to tetanize fish and kill a high percentage of the catch (Lamarque 1990). Continuous DC electrofishing is believed to be the least damaging form because fish are attracted to the anode and are immobilized by galvanonarcosis, not tetany. The effects of pDC electrofishing are thought to be intermediate between AC and DC because fish are immobilized by tetany (Lamarque 1990). In the present study, pDC electrofishing did not induce any mortality. Similarly, Horak and Klein (1967), Maxfield et al. (1971), and Whaley (1975) reported no mortality following pDC electrofishing. In contrast, mortalities of 2–11% of fish captured have been reported with AC electrofishing gear (Pratt 1954; Hudy 1985). The lack of mortality in our study, therefore, supports the hypothesis that pDC is less damaging than AC.

Spinal misalignment induced by pDC electroshock was only observed in large (~600 g) rainbow trout treated with 600 V for either 20 or 40 s (Table 1). These results support the earlier conclusion that larger fish are influenced more quickly and by relatively smaller voltages than smaller fish (Halband 1967; Bohlin et al. 1989), since they experience greater head to tail voltages (Novotny 1969; Ellis 1975; Reynolds 1983). The only previous study of spinal injury resulting from pDC electroshock was conducted by Sharber and Carothers (1990). Surprisingly, they found damage involving an average of eight vertebrae in 50% of the rainbow trout examined. Such high percentages have never before been reported, even following treatment with AC gear (Hauck 1949; McCrimmon and Bidgood 1965; Hudy 1985). An explanation of the differences in results is unclear, but they may be partially attributed to differences in water conductivity (600–800 versus 240–270 μmho/cm with voltages of 260 and 200 V, respectively) and/or fish size (300–560 versus 130–430 mm) between this and the present study.

Thus, we can categorize the electroshock treatment used in the present study as being above the threshold for immobilization, but below that causing physical damage and mortality.

### Physiological Effects of Electroshock

The hypothesis that pDC electrofishing is more disturbing to fish than continuous DC (Lamarque 1990) is also strongly supported by the comparison of plasma cortisol, glucose, and whole-blood lactate levels in our study with changes previously reported for continuous DC (Table 2). Of the previous studies, ours is most comparable (in terms of species, water quality, and voltage) with that of Schreck et al. (1976). Both cortisol and lactate elevations following pDC are at least double those reported by Schreck et al. (1976) for continuous DC and are generally more severe than all previous continuous DC electrofishing studies (Woodward and Strange 1987; Mesa and Schreck 1989).

Similarly, the elevation in plasma glucose is higher in fish treated with pDC than with continuous DC (Schreck et al. 1976; Woodward and Strange 1987). In fact, the stress response elicited by pulsed DC electrofishing is similar in magnitude to other acute stressors in salmonids such as exhaustive exercise (e.g., peak glucose levels of 5 mmol/L; Ristori and Laurent 1985; Milligan and Wood 1986a) and handling (e.g., maximum cortisol: 45–140 ng/mL; Barton et al. 1980, 1985, 1986; maximum glucose: 5–6 mmol/L; Barton et al. 1986).

Catecholamines were also mobilized immediately following electroshock (Fig. 2A and 2B). The stimulus for their release is likely the perception of stress or pain acting through sympathetic innervation of the chromaffin tissue (Mazeaud and Mazeaud 1981), but the blood acidosis (Tang and Boutilier 1988) and a decrease in blood oxygen content (Perry et al. 1989; Fievet et al. 1990) from the apnea may have also contributed. No other studies, to our knowledge, have examined catecholamine concentrations following electroshock. However, a number of studies have reported epinephrine and norepinephrine values after bouts of exhaustive exericse (2–10 min in duration). While reported levels are highly variable (increases in epinephrine of 27–210 nmol/L and norepinephrine of 26–75 nmol/L; Butler et al. 1986; Primmett et al. 1986; Milligan and Wood 1987; Tang and Boutilier 1988), catecholamine mobilization following electroshock was generally of a smaller magnitude: 1/3–1/20 that of exhaustive exercise.

The maximum increase in blood lactate observed in this study was double that of the largest reported value for continuous DC (Table 2) (Schreck et al. 1976; Mesa and Schreck 1989). In fact, several studies have reported no elevations in lactate (reviewed by Lamarque 1990). The more pronounced elevation of lactate with pDC was probably not solely a result of impaired ventilation, as hypothesized by Schreck et al. (1976), since the apnea in this study was no more prolonged than in previous continuous DC studies. Instead, the muscular tetany unique to pDC is likely an important contributing factor.

In addition to the metabolic acidosis, fish experienced a respiratory acidosis, which exacerbated the overall reduction in arterial pH. The respiratory acidosis may have been a result of gas exchange impairment during apnea (Schreck et al. 1976), but also could have been simply due to $H^+$ titration of $HCO_3^-$. Plasma pH recovered quickly from the acidosis, slightly overshooting resting pH values at 4 h due to overcompensation (Milligan and Wood 1986a).

The metabolic and respiratory acidoses attributing to the depression in pH induced by electroshock were similar to those induced by 2–3 min of exhaustive exercise (pH reduction of 0.3 unit; Butler et al. 1986), yet were slightly less than the 0.3- to 0.5-unit reduction in arterial pH resulting from 5–6 min of exhaustive exercise (Holeton et al. 1983; Turner et al. 1983; Wood et al. 1983; Milligan and Wood 1986a, 1986b; Primmett et al. 1986; Milligan and Wood 1987; Tang and Boutilier 1988; Ferguson and Tufts 1992).

### Combined Effects of Electroshock and Air Exposure

Following electrofishing, fish are often exposed to air for extensive periods of time before being released. During this time, the gill lamellae collapse, inhibiting gas exchange (Boutilier 1990; Ferguson and Tufts 1992). Reduction or elimination of oxygen uptake and carbon dioxide excretion during air exposure may have led to the slightly elevated lactate and acid–base disturbances observed following 1 min of treatment (Fig. 3A–3D). Few studies have examined the physiological effects of air exposure alone. However, during severe hypoxia in water (comparable with air exposure), glycolytic rates are elevated resulting in increased muscle lactate (Dunn and Hochachka 1986) and reduced pH (Smith and Heath 1980; Thomas and Hughes 1982; Thomas et al. 1986). This hypoxic stress also tends to increase plasma catecholamine levels (Butler et al. 1978; Tetens and Christensen 1987). However, the combined treatment of electroshock and 1 min of air exposure did not significantly elevate any of these parameters above that of electroshock alone.

The lack of a significant further elevation in plasma catecholamines, cortisol, and glucose as a result of air exposure indicates that the combined stressors were not additive. Also, there was a surprisingly small additional accumulation of lactate (+2 mmol/L) in light of the likely absence of branchial oxygen uptake during air exposure. In contrast, Ferguson and Tufts (1992) showed that 1 min of air exposure following exhaustive exercise elevated blood lactate by a much greater amount, at least 10 mmol/L above that of exercise alone. The lack of additional lactate accumulation following pDC electroshock suggests, in fact, that metabolism may have been inhibited for a short time. The nature of this postelectroshock metabolic depression is unclear but may be related to the anaesthetic effects of electrical current (Summerfelt and Smith 1990). Mesa and Schreck (1989) similarly showed no elevation in the lactate levels in cutthroat trout (*Oncorhynchus clarki*) following treatment with continuous DC gear, anesthetization, weighing, measuring, and marking over treatment with continuous DC electroshock only.

Despite these physiological disturbances, the present study has shown that rainbow trout can survive up to 4 min of exposure to air following pDC electroshock. Barrett and Grossman (1988) similarly found no mortality in mottled sculpin (*Cottus bairdi*) during the week following DC electroshock and approximately 1 min of handling. Ferguson and Tufts (1992), however, reported elevated mortality in exercised rainbow trout following periods of only 30–60 s of air exposure. Again, this may be due to differences in metabolic rates following treatment. In addition, Ferguson and Tufts' study was conducted on catheterized fish, which in itself is stressful, and may have accounted for the increased mortalities.

Overall, our results suggest that pDC electrofishing is a suitable procedure for capturing fish in the field that does not normally produce any skeletal damage. In addition, it evokes physiological disturbances in blood lactate and pH that are comparable with brief (2–3 min) periods of forced exercise. These disturbances are either comparable or less severe than what a fish might experience with angling capture and release. Furthermore, we show that the residual effects of electroshock may actually reduce the physiological effects of handling of fish out of water. Nonetheless, to ensure the health of fish being returned to the wild, handling following electroshock should be minimized.

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# Effects of Electroshock, Air Exposure, and Forced Exercise on Swim Performance in Rainbow Trout (*Oncorhynchus mykiss*)

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Swim performance of rainbow trout (*Oncorhynchus mykiss*) following forced exercise and pulsed DC electroshock both with and without subsequent air exposure was examined. Swim stamina was assessed by exercising fish at 39 cm/s until exhausted. Swim performance was impaired following all treatments. Increasing durations of air exposure (0–4 min) after electroshock resulted in progressively prolonged durations of swimming impairment (from 1 to 6 h). Electroshock reduced swimming stamina in a virtually identical fashion to that of 5 min of forced exercise. By comparison with the existing literature on the physiological effects of forced exercise, the impairment of swim performance most likely resulted from a reduction in intracellular pH.

On a examiné la performance à la nage de la truite arc-en-ciel (*Oncorhynchus mykiss*) après un exercice forcé et un choc électrique de courant continu avec ou sans exposition subséquente à l'air. On a évalué l'endurance à la nage du poisson en le forçant à nager à 39 cm/s jusqu'à ce qu'il soit épuisé. La performance à la nage a été diminuée après tous les traitements. Une augmentation de la durée de l'exposition à l'air (0–4 min) après le choc électrique s'est traduite par une prolongation progressive de la durée de l'incapacité à nager (de 1 à 6 h). Le choc électrique a réduit l'endurance à la nage d'une façon essentiellement identique à un exercice forcé de 5 min. En comparant les données obtenues avec celles de la littérature sur les effets physiologiques de l'exercice forcé, on peut affirmer que la diminution de la performance à la nage résulte fort probablement d'une diminution du pH intracellulaire.

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Electrofishing is a capture technique that is used extensively worldwide. Following capture, fish are often exposed to air for extensive periods for processing before being released. However, sluggish swimming is widely reported following electrofishing (Hauck 1949; Horak and Klein 1967; Curry and Kynard 1978; Hudy 1985; Mesa and Schreck 1989), and handling tends to increase the severity of the effect (Mesa and Schreck 1989). Reduced swim performance impairs predator avoidance (Curry and Kynard 1978) and thus increases the chance of mortality. However, the duration and magnitude of inhibited swim performance resulting from electroshock have not been critically examined.

The objective of this study was therefore to examine swimming stamina following pulsed DC (pDC) electroshock and handling. Stamina was evaluated by a procedure similar to that employed by Nahhas et al. (1982), and handling in the field was simulated by using air exposure of various durations (0–4 min). In a previous study (Mitton and McDonald 1994), we established that the physiological effects of electroshock were quantitatively similar to those produced by 2–3 min of exhaustive exercise. Consequently, we also examined the effects of forced exercise on swim performance. Finally, the effects of all treatments on anaerobic activity were assessed by measuring changes in whole-body lactate content.

## Materials and Methods

### Experimental Animals

Juvenile rainbow trout (*Oncorhynchus mykiss*) (weight 3–12 g; length 6–12 cm) were obtained from Rainbow Springs Trout Farm in Thamesford, Ont. Fish were held in well-aerated, 400-L circular tanks supplied continuously with dechlorinated municipal tap water. Fish were fed daily with commercial trout pellets (Martins Feed Mills, Elmira, Ont.). One day before treatment, fish were acclimated to the test tank (2.4 × 0.6 m) and were not fed during this time. The temperature and conductivity were 14 ± 2°C and 255 ± 15 μmho/cm, respectively.

### Experimental Protocol

In all electroshock trials, fish were confined in a volume of 56 L where they were at least 0.3 m from the cathode and 1.2 m from the anode (see Mitton and McDonald 1994 for details). Electrofishing was conducted with a Smith–Root VII pDC backpack electrofisher at 200 V for a 20-s duration, with the pulse duration and frequency set at 5 ms and 60 Hz, respectively. These frequency and pulse duration settings are commonly used to capture fish in field studies in hard waters typical of southern Ontario (R. Dalziel, Ontario Ministry of Natural Resources, 90 Shepard Ave. E., 5th Floor, North York, ON M2N 3A1, personal communication). Immediately after electroshock, fish were netted and placed into

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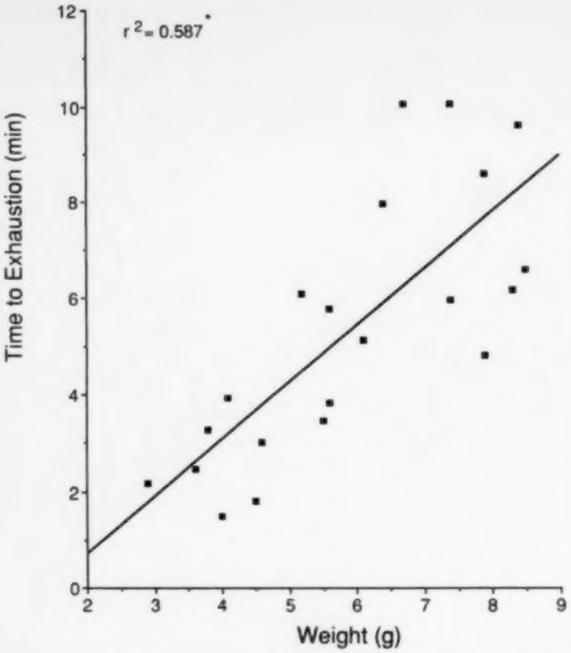

FIG. 1. Time to exhaustion of control fish in relation to body weight ($r^2 = 0.587$). $T = 14 \pm 1$°C; * significant correlation ($p < 0.05$).

a container lined with moistened paper towels. Following 0, 1, 2, or 4 min of air exposure, fish were returned to the test tank.

For forced exercise, fish were transferred (five or six at a time) to a 10-L cylindrical chamber containing 6 L of water and forced to swim by manually chasing them for 5 min.

### Swim Performance Test

Swim performance measurements were conducted in a 150-L recirculating swim tunnel (Research Instruments Mfg. Co., Guelph, Ont.), similar in design to that of Farmer and Beamish (1969). Current was generated by a 3-hp (1 hp = 743 W) motor powering an impellor capable of producing water velocities up to 60 cm/s. A cooling jacket of anodized aluminum was used to maintain water temperature at 14 ± 1°C. Following treatment, trout ($N = 8$–12 per group, five groups) were transferred and acclimated to the swim tunnel for 30 min at a water speed of 12 cm/s (1.1–2.0 body lengths (BL)/s; mean = 1.5 BL/s). At the end of the acclimation period, fish were exposed to a sudden increment in velocity. Through trial and error, a fixed velocity of 39 cm/s was found to produce exhaustion in the time desired (1.5–8.6 min). This velocity represented a swimming speed of 3.5–6.5 BL/s for the fish used, with the average being 4.5 BL/s. Exhaustion was defined as the time at which the fish was forced against the downstream screen and could not be further stimulated to swim by prodding. Fatigued fish were immediately removed unharmed by siphoning with a 4-cm-diameter tube. This procedure permitted the remaining fish to continue swimming relatively undisturbed.

For each fish the time to exhaustion and total length (±0.5 cm) and weight (±0.1 g) were recorded. Swim performance was determined at 0.5, 1, 2 and 4 h following treatment and at 6 h for treatment groups not fully recovered by 4 h. Controls were treated the same as test fish, but were not electroshocked or exercised. Controls were transferred from the holding tanks to the swim tunnel and given the same 30-min period to acclimate as the test fish. Therefore, the performance of the control fish, which only had 30 min to recover from transfer, was probably not truly representative of unstressed resting fish.

### Whole-Body Lactate

At two times following treatment (0.5 and 4 h), trout were carefully netted to minimize struggling and immediately stunned with a blow to the head. Fish were then transferred to a bath of liquid nitrogen (−170°C) and freeze-clamped with metal plates. All fish per group ($N = 8$–11) were sampled within a 5-min period. Total lengths and weights were recorded and whole fish then powdered with a liquid nitrogen cooled mortar and pestle. Eight millilitres of 8% perchloric acid (PCA) was immediately added to the powder and mixed for 2 min on ice with a tissue homogenizer (Janke and Kunkel, Ultra-turrax®). The resulting solutions were centrifuged for 4 min and the supernatant was drawn off and frozen at −70°C for later analysis. After thawing, aliquots of supernatant (0.2 mL) were further diluted 1:3 with 8% PCA. The PCA extracts were then assayed for lactate using the lactate dehydrogenase method with Sigma reagents. Lactate values were corrected for tissue weight and dilution and expressed as micromoles per gram wet tissue mass.

### Statistical Analysis

All values are expressed as mean ± 1 standard error of the mean (SEM). Significance was determined by the unpaired Student $t$-test ($p < 0.05$).

## Results

### Swim Performance Test

Body masses ranged from 2.9 to 8.4 g in the fish used, and the time to exhaustion varied in a linear fashion with body mass ($r^2 = 0.587$; Fig. 1). To correct for the effects of size on swim performance, time to exhaustion was expressed per unit body weight (Nahhas et al. 1982). With this correction, time to exhaustion averaged 52.3 ± 3.9 s/g in control (i.e., before treatment) fish ($N = 21$; Fig. 2).

### Effects of Treatments on Swim Performance

All treatments produced impaired performance in comparison with the control fish. Electroshocked fish exhibited a 32% decrease in swim performance at 0.5 h following treatment and then a further impairment to 53% of control by 1 h (Fig. 2A). Swim performance had fully recovered by 2 h after the cessation of electroshock. Fish that were exposed to air for 1 min following electroshock similarly experienced a depression in swim performance at 0.5 h with a maximum impairment of 51% of control 1 h after treatment. However, swim performance in these fish did not recover until 4 h following treatment (Fig. 2B). Electroshocked fish that were air exposed for 2 min exhibited a swim performance depression similar to that induced by electroshock alone, by 0.5 h after treatment. By 1 h, these fish exhibited a further impair-

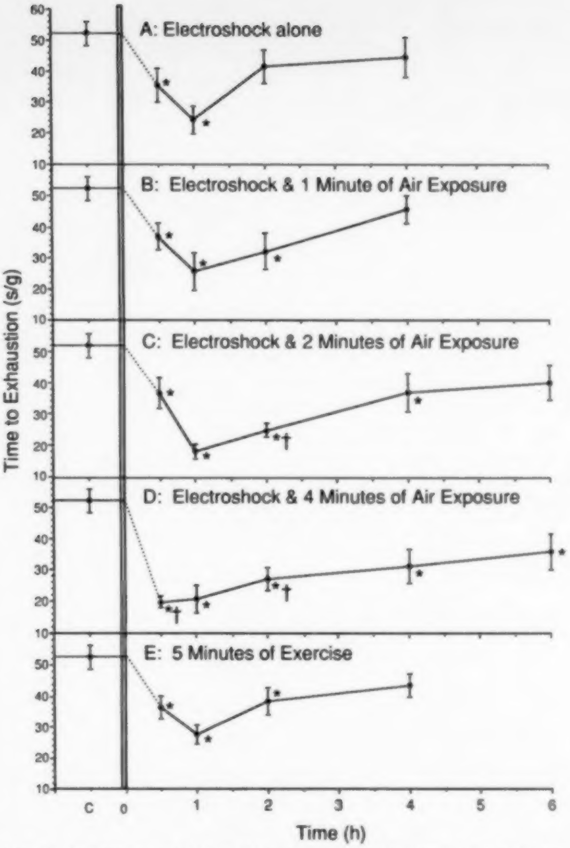

FIG. 2. Influence of (A) a 20-s electroshock alone, (B) electroshock and 1 min of air exposure, (C) electroshock and 2 min of air exposure, (D) electroshock and 4 min of air exposure, and (E) a 5-min bout of exhaustive exercise on swim performance (shaded bar indicates treatment period). Swim performance is expressed in time to exhaustion per gram of body weight. All values are expressed as mean ± 1 SEM ($N$ = 8–22); $T$ = 13 ± 1°C; * significant difference from resting ($p < 0.05$); † significant difference from electroshocked fish ($p < 0.05$).

ment in swimming of 65% relative to control values, with full recovery not occurring until 6 h posttreatment (Fig. 2C). Treatment with 4 min of air exposure after electroshock induced a 62% reduction in swim performance 0.5–1 h following treatment, and recovery required longer than 6 h (Fig. 2D). Exercised fish also experienced impaired swimming with a pattern similar to that induced by electroshock alone (Fig. 2A and 2E): an initial impairment of 47% followed by a further reduction in performance at 1 h to 53% of control. Recovery of swim performance occurred by 4 h following treatment.

### Whole-Body Lactate

Following all treatments, whole-body lactate was significantly elevated above control concentrations of 1.4 ± 0.1 μmol/g at 0.5 h and had recovered by 4 h. At both 0.5 and 4.0 h posttreatment, the lactate content in electroshocked fish (5.6 ± 0.6 and 0.8 ± 0.1 μmol/g, respectively) was similar to that of exercised fish (5.1 ± 0.7 and 1.1 ± 0.3 μmol/g, respectively; Fig. 3). Additionally, at 0.5 h following treatment, whole-body lactate tended to rise in proportion to the amount of air exposure, but the trend was not significant ($r^2 = 0.082$; Fig. 3). This trend was amplified by 4 h and became significant ($r^2 = 0.383$, $p < 0.05$; Fig. 3). Even though the trend was significant, there was not a significant difference in whole-body lactate between fish electroshocked only and those subjected to the most severe electroshock and air exposure treatment (4 min).

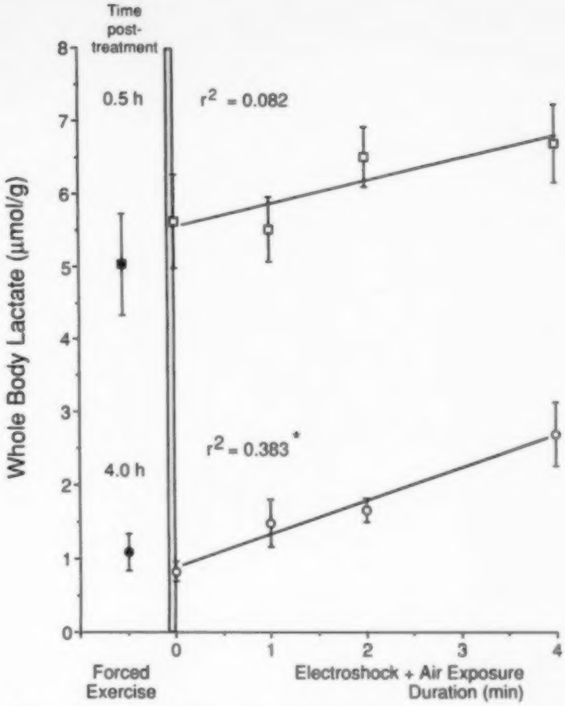

FIG. 3. Influence of exercise (solid symbols) or electroshock (open symbols) with and without subsequent air exposure on whole-body lactate at 0.5 h ($y = 5.54 + 0.31x$) and 4 h ($y = 0.88 + 0.45x$) after treatment, respectively (shaded bar indicates the electroshock treatment). All values are expressed as mean ± 1 SEM ($N$ = 8–11); $T$ = 8 ± 2°C; * significant correlation ($p < 0.05$).

## Discussion

Swim performance has been measured in at least three different ways in previous studies (reviewed in Beamish 1978): by measures of burst performance, by sprint testing, as in the present study, or by measures of critical swimming speed ($U_{crit}$). $U_{crit}$ determinations are the most common measure of swimming ability, but this test assesses aerobic performance, which is not a key component in challenges such as predator avoidance. In addition, during the long periods (2–4 h) required to administer $U_{crit}$ tests, fish would be recovering from the acute electroshock and exercise treatments. Although rapid burst performance tests closely simulate natural challenges, such as predator avoidance, the short time required to complete the tests (<20 s, and often <1 s; Beamish 1978) and the dependence on training make subtle differences in performance difficult to resolve. Therefore, the sprint performance test we employed, which measures a combination of aerobic and anaerobic swimming capacity over 2–10 min, can be considered a good compromise, simulating responses to predators while permitting resolution of treatment differences. Such sprint testing has been used previously to assess differences among strains, effects of domestication or training (Reimers 1956; Vincent 1960; Hammond and Hickman 1965; Horak 1972; Nahhas et al. 1982; Houlihan and Laurent 1987), as well as recovery from physiological disturbances associated with transport (Maule et al. 1988).

This study clearly shows that swim performance was impaired by electroshock in a virtually identical fashion to that caused by forced exercise, with swim performance being depressed 0.5 h following treatment and maximum inhibition occurring at 1 h. Furthermore, impairment was progressive with increasing duration of air exposure following electroshock.

The impaired performance is undoubtedly related to muscle fatigue. Although a variety of physiological and biochemical factors may contribute to the development of fatigue, there are three prominent causes: (1) the depletion of energy stores within the muscle necessary for muscle contraction (e.g., ATP, creatine phosphate, and glycogen; Guyton 1986; Hill and Wyse 1989; Scarabello et al. 1991b), (2) the development of an extracellular acidosis, and (3) the development of an intracellular acidosis. An extracellular acidosis can lead to fatigue by impairment of hemoglobin oxygen transport (Hill and Wyse 1989), while an intracellular acidosis inhibits anaerobic enzyme activity (Jones and Randall 1978; Blanchard and Solaro 1984; Guyton 1986; Westerblad et al. 1991). These factors have been examined following exhaustive exercise, although not after electroshock. Since pDC electroshock appears to induce physiological disturbances similar to those from exercise (Mitton and McDonald 1994), one can speculate on the proximate cause(s) of the impaired swimming performance in this study.

Although muscle metabolic substrate levels are reduced following bouts of exhaustive exercise (Milligan and Wood 1986b; Scarabello et al. 1991a, 1991b), the maximal substrate depletion is immediate whereas impairment of swim performance continues to develop to 1 h. Furthermore, the extracellular acidosis induced by either electroshock (Mitton and McDonald 1994) or exhaustive exercise (Holeton et al. 1983; Wood et al. 1983; Milligan and Wood 1986a; Tang and Boutilier 1988; McDonald et al. 1989; Tang et al. 1989; Ferguson and Tufts 1992) is also immediate, with significant recovery by 1 h.

In contrast, the intramuscular acidosis, while developing immediately following exhaustive exercise, continues to develop until 1 h posttreatment, with full recovery requiring from 8 to 12 h (Milligan and Wood 1986a, 1986b; Tang and Boutilier 1991). As this pattern is comparable with the progressive decline and recovery of swim performance after electroshock (Fig. 2), we suggest that the decline in intramuscular pH may have been the principal cause of impaired swim performance rather than either an excellular acidosis or substrate depletion. We further suggest that air exposure either further depressed intramuscular pH or impaired its recovery to explain the progressive impairment in swim performance that we observed with increasing air exposure. However, further work on muscle acid–base balance and metabolite levels following electroshock and air exposure will be needed to resolve this issue, especially since variations in whole-body lactate (a putative correlate of intracellular pH) among treatments in this study were too small to account for the significant differences in swim performance.

### Ecological Relevance

The 2-h inhibition of swim performance experienced by electroshocked rainbow trout (Fig. 2) was much less prolonged than previously reported performance effects determined via a modified $U_{crit}$ test by Horak and Klein (1967). Those authors reported reduced performance 24 h after treatment with pDC electrofishing gear. However, 39% of the rainbow trout in their study exhibited areas of internal bleeding, which is often associated with spinal damage (Lamarque 1990). Thus, Horak and Klein's study examined the chronic deterioration in swim performance induced by physical damage, while our study assessed the acute impairment of swim performance induced by physiological disturbances.

Both the magnitude and duration of swim performance impairment increased with the duration of air exposure (Fig. 2A–2D). Therefore, air exposure during postelectroshock handling has the potential to be more detrimental physiologically than the capture technique itself, since throughout a prolonged period of impaired swim performance, predator avoidance ability of the fish is likely also reduced. Thus, when electrofishing is used in the field to collect juvenile or small fish, with the intent of returning them to the wild, air exposure during netting and processing should be minimized. Similarly, Barrett and Grossman (1988) and Mesa and Schreck (1989) concluded that handling following DC electrofishing may be more detrimental than the capture. Also, Ferguson and Tufts (1992) recommended that handling of angled fish before release be minimized.

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# An Index of Biotic Integrity (IBI) for Fish Assemblages in the Littoral Zone of Great Lakes' Areas of Concern

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Karr's Index of Biotic Integrity (IBI) approach provides a biological measure of ecosystem health using a wide spectrum of metrics which can be extracted from fish catch data obtained using standardized methods. Extensive electrofishing surveys of littoral fish assemblages, conducted in three Great Lakes' Areas of Concern, provided the basis for developing a lacustrine IBI that was 12 metrics of three broad types: (i) species composition, (ii) trophic composition, and (iii) abundance and condition. In contrast with lotic IBIs where diversity and abundance metrics have mostly been used, several biomass metrics were adopted to accommodate the large size range of lentic fishes. The variability of repeated measures was low enough to allow valid testing of intertransect differences with three to five samples per transect. Comparisons among survey areas showed significant differences consistent with the varying levels of ecosystem degradation. Analyses of mean IBI values with measures of submerged vegetation density and cover by transect produced significant positive correlations. This IBI developed for the Great Lakes' littoral zone, both by design and by demonstrated correlations, integrates the effects of four main factors influencing fish assemblages and hence revealing ecosystem health: exotic fishes, water quality, physical habitat supply, and piscivore abundance.

L'indice d'intégrité biotique (IIB) de Karr nous fournit un outil biologique pour mesurer la santé de l'écosystème grâce à une gamme étendue de mesures que l'on peut extraire de données sur les captures de poissons obtenues à l'aide de méthodes normalisées. Des études exhaustives des rassemblements de poissons littoraux à l'aide de la pêche à l'électricité, effectuées dans les secteurs préoccupants des Grands Lacs, nous ont permis d'élaborer un IIB lacustre qui fait appel à 12 mesures appartenant à trois types généraux : i) composition des espèces, ii) composition trophique et iii) abondance et conditions. Contrairement aux IIB lotiques où des mesures de diversité et d'abondance ont été utilisées la plupart du temps, plusieurs mesures de la biomasse ont été adoptées pour tenir compte de la gamme étendue de tailles des poissons lénitiques. La variabilité des mesures répétées était suffisamment faible pour permettre une évaluation valide de différences entre transects au moyen de trois à cinq échantillons par transect. Les comparaisons effectuées entre les secteurs étudiés ont montré des différences significatives compatibles avec les divers degrés de dégradation de l'écosystème. Une analyse des valeurs moyennes d'IIB avec des mesures de la densité et de la couverture de végétation submergée par transect a donné des corrélations positives significatives. L'IIB élaboré pour la zone littorale des Grands Lacs, tant du point de vue de sa conception que des corrélations démontrées, intègre les effets des quatre principaux facteurs affectant les rassemblements de poissons et ainsi révèle la santé de l'écosystème : poissons exotiques, qualité de l'eau, habitat physique et abondance de piscivores.

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> Examine each question in terms of what is ethically and aesthetically right, as well as what is economically expedient. A thing is right when it tends to preserve the integrity, stability and beauty of the biotic community. It is wrong when it tends otherwise. (*Leopold 1949*)

In the late twentieth century, western humans have, perhaps belatedly, begun to heed the advice of Leopold and others, and environmental scientists have begun to devise measures of integrity, stability, and even beauty as guides to the "rightness" of things. In the Great Lakes of North America, the need for an "ecosystem approach", an integrative holistic balancing of social, economic, and environmental considerations (Christie et al. 1986), was emphasized in the 1978 renewal of the Great Lakes Water Quality Agreement (GLWQA). In 1987, that approach became the purpose in a further renewal of the agreement: "to restore and maintain the physical, chemical, and *biological integrity* of the waters of the Great Lakes Basin Ecosystem" (our italics for emphasis). For ecologists, the challenge has been to devise measures and indices of biological integrity.

In 1986, the International Joint Commission (IJC) called on Canada and the United States to prepare "Remedial Action Plans" (RAPs) for 42 (now sadly 43) "Areas of Concern" (AOCs) around the Great Lakes, requiring that the RAPs reflect the ecosystem approach. Sites are designated an AOC if one or more of 14 identified beneficial uses are impaired (cf. Annex 2, Revised Great Lakes Water Quality Agreement of 1978, 1987). These beneficial uses are divisible between those related to intrinsic structures, states, and functions of ecosystems and those related to human uses of ecosystems.

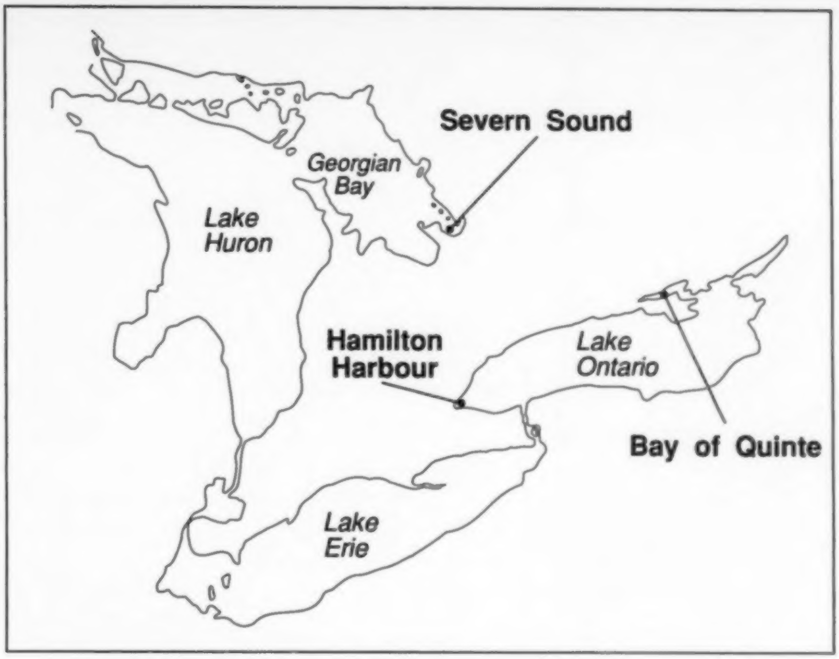

FIG. 1. Lower Great Lakes region showing the location of the three Areas of Concern: the Bay of Quinte and Hamilton Harbour on Lake Ontario and Severn Sound on Georgian Bay.

Among the 14 beneficial uses, five refer directly to fish ((1) restrictions on consumption, (2) tainting, (3) degradation of populations*, (4) tumours and other deformities*, (14) loss of habitat*) and another four, possibly more, refer to components of fish habitat ((6) degradation of benthos*, (8) eutrophication*, (13) phytoplankton and zooplankton*, and (11) aesthetics) (asterisks mark the intrinsic ones). The fish-related uses represent the majority of the intrinsic ecosystem properties, and thus, fish ought to be useful in selecting targets and criteria for assessing, restoring, and managing aquatic ecosystems. Fishes and their habitat requirements are also a primary target of efforts to "clean up" the Great Lakes.

The RAP process has created a need for operational definitions of all beneficial uses justifying the listing, and delisting, of sites as AOCs. A general question emerges, i.e., which measures of what ecosystem features should be used to determine if a beneficial use is impaired or restored? Ecosystem health can be measured directly using the biota present or indirectly using physicochemical features of their habitats. The latter approach was the tradition among water quality managers on the Great Lakes until the ecosystem approach emerged. The tendency has been to try to measure ecosystem health with biotic indices *or* abiotic surrogates but the approaches cannot be used in isolation. Abiotic surrogates must be validated by reference to biotic measures and the causality of various biotic states must be known. Logistic, and financial, constraints often preclude adequate biotic assessments, forcing reliance on simpler but less reliable abiotic measures. Fish should have a pivotal role in many potential listing/delisting criteria. Fish assemblage abundance and composition, species-specific targets, fish health, and fish contaminant burdens are among the criteria currently in use or being considered.

Karr (1981) developed the Index of Biotic Integrity (IBI) as a rapid, cost-effective means of assessing the "health" of stream ecosystems, using standardized assessments of fish assemblages and a set of metrics reflecting the broad array of abiotic and biotic factors influencing the fish. This and subsequent approaches emphasize a preference for direct/biotic over indirect/physicochemical assessment of ecosystem health. Karr and Dudley (1981) defined biological integrity as

> the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition and functional organization comparable to that of natural habitat of the region.

This definition applies where the phrase "biological integrity" is used in the statement of purpose for the GLWQA. Many riverine applications and extensions of Karr's IBI concept have followed Karr's initial work (cf. Fausch et al. 1990 and Karr 1991 for reviews). Fausch et al. (1990) reviewed several approaches to using fish assemblage data as the basis for indices of ecosystem degradation and argued that the IBI, a composite index, offered the best balance of advantages and disadvantages. Indices like IBIs are not meant to be explanatory models of ecological phenomena but rather to provide a means of conveying measures and knowledge of aspects of ecosystem health to a broader, often nontechnical audience which will include decision-makers.

From 1988 to 1990, our research group undertook an extensive study of fishes and their habitat in the littoral zones of three Great Lakes' AOCs. We accumulated a large set of fish assemblage and habitat data with the primary intention of quantitatively relating biotic and abiotic features. Our database provided an opportunity to develop and evaluate an IBI for littoral fish assemblages which could provide a defensible, reproducible measure of impairment or restoration of fish-related intrinsic uses of the ecosystem. The fact that all data were collected within AOCs may seem inconsistent with the established IBI methodology which requires that samples from unperturbed, undegraded, unaltered sites be used to establish reference bench marks. However, all sites within any AOC are not equally affected by listed use impairments. The designation of AOCs tended to be geographically arbitrary and was not based on detailed spatial delineation of impairments. For instance, the whole of Severn Sound, one of the sites studied, is designated an AOC although it is clear the greatest concern is for Penetang Harbour, while many other subareas show no noticeable impairment. We have assumed that some sites, although within the bounds of an AOC, have representative healthy fish assemblages.

The objectives of this paper are to (i) derive an IBI applicable to samples of fish assemblages collected by electrofishing in littoral habitats of Great Lakes' AOCs, (ii) analyze the properties of the index and its constituent metrics, and (iii) assess the performance and value of the IBI as an indicator of water quality and habitat conditions for fish both within and between the three AOCs: Bay of Quinte, Hamilton Harbour, and Severn Sound (Fig. 1).

## Materials

### Study Areas on the Great Lakes

Despite sharing IJC-imposed impairment designations, the three AOCs targeted in our studies of littoral fish assemblages cover a wide range of ecosystem health. The number of impaired beneficial uses and the degree of impairment vary considerably. All parts of each AOC are not equally impaired. The five survey areas span a range of ecosystem impairment and degradation. They can be ranked by overall impairment from the greatest number (1) through lowest degree (5) as follows: (1) Hamilton Harbour, (2) the Bay of Quinte and, within Severn Sound, (3) Penetang Harbour, (4) Hog Bay, and (5) Matchedash Bay.

Hamilton Harbour, among the most degraded of the 43 AOCs, is a small body of water (2150 ha, mean depth 13.0 m) connected by a narrow ship canal at the western end of Lake Ontario. The harbour's catchment is highly urbanized and industrialized. Indeed, extensive land-filling in conjunction with the historical development of large industries and habour facilities has greatly reduced the size of the harbour and eliminated most of the vegetated littoral habitat. Although substantial progress has been made towards reducing nutrient and contaminant loadings over the last 20 yr, the harbour remains highly eutrophic with a legacy of highly contaminated sediments and fish populations with high incidence of external anomalies and internal cancers (COA 1989; Rodgers 1992). Much of the harbour's outflow comes from a single Hamilton sewage treatment facility. Fishery opportunities in Hamilton Harbour are minimal due to the very low levels of piscivores like northern pike[1] and largemouth bass. Fish biomass is dominated by large common carp, an introduced species, while numbers are dominated by two other exotic species, alewife and white perch.

The Bay of Quinte is a long narrow bay connecting to the eastern outlet basin of Lake Ontario. The upper Bay is large and shallow (13 640 ha, mean depth 4.2 m). The many papers in Minns et al. (1986) provide an extensive assessment of eutrophication in the Bay of Quinte. The Bay has a large watershed, and runoff flushes the upper Bay 12 times per year on average. The upper Bay has been most affected by eutrophication due to sewage inputs from adjacent urban centres. Prior to a 50% reduction of point-source phosphorus inputs in 1978 the upper Bay was considered hypereutrophic. After 1978, the upper Bay was still eutrophic. The eutrophication has reduced water clarity and diminished the extent and abundance of submerged macrophyte communities. Coincident, and most likely unrelated, to the decline in phosphorus inputs, walleye began a major recovery producing a substantial sport fishery. Through the 1960s and 1970s, the fish community had been dominated by alewife and white perch. White perch supported a large portion of the commercial fishery in the lower Bay until severe cold temperature mortalities sharply reduced the population in the winter of 1977–78. Piscivores were a small part of the assemblage until the walleye recovery. Other piscivores such as northern pike and bass species have not recovered probably because the macrophytes which provide important habitat for them have not expanded. Overall the fish assemblage must still be considered degraded as long as the diversity of top piscivores remains low. Although bottom sediments in some areas and the large members of some fish species have elevated metal and organic contaminant levels, contaminants and toxicants are not considered a severe problem in the Bay of Quinte (BOQ 1989).

Severn Sound is in the southeast corner of Georgian Bay and consists of a series of bays opening into the sound. The sound lies on the boundary between sedimentary and Precambrian granitic formations. The three bays we have studied are on the southern sedimentary side: Penetang Bay, Hog Bay, and Matchedash Bay. Severn Sound is listed as an AOC for three main reasons: (i) eutrophication particularly in Penetang Bay where a combination of intense development and slow flushing have degraded water quality, (ii) decline in some sport fish populations, particularly walleye, and (iii) elevated contaminant levels in larger sized specimens of selected fish species (COA 1988). Hog Bay is a more open exposed system, while Matchedash is more enclosed with abundant emergent and submerged macrophyte communities.

### Source and Characteristics of Fish and Habitat Data

In 1988, 1989, and 1990, we conducted an extensive program sampling fish assemblage and their habitats in the littoral zones of the three AOCs. The standardized fish sampling program is described in detail in Randall et al. (1993). In brief, a Smith–Root electrofishing boat was used to sample 100-m transects which ran parallel to shore approximately following the 1.5-m depth contour. Each transect was selected to represent a fairly homogeneous habitat type and, in each survey area, transects were selected to span the range of nearshore habitat types available with several representatives of each type. The fish catch from each transect sampling was sorted by species and the number and total wet biomass of each species determined.

This program produced seven groups of data (Table 1); data sets were obtained for the Bay of Quinte in 1989 and

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[1] Latin names for all freshwater fish species mentioned in the text are given with the corresponding common names in Table 1.

1990, for Hamilton Harbour in 1988 and 1990, and for three portions of Severn Sound (Penetang Harbour, Hog Bay, and Matchedash Bay) in 1990. The number of different transects varied from 12 to 48 in different areas. In the Bay of Quinte, transects sampled in 1990 were a subset of those sampled in 1989. In Hamilton Harbour, the 40 transects sampled many times in 1988 were revisited in 1990 along with another eight sites in the vicinity of wetlands in Cootes Paradise at the western end of the harbour. In all the 1990 data sets, transects were sampled one to three times during the summer. In 1988, Hamilton Harbour transects were sampled 7–11 times. In 1989, all Bay of Quinte transects were sampled once and two a second time in August.

Overall, 45 fish species, including hybrids, were encountered in our electrofishing surveys (Table 1). Total species richness per data set ranged from 17 to 30. Among a total of 654 samples, including 13 where no fish were caught, species' frequencies of occurrence range from 1 to 393. Fourteen species occurred less than 10 times, five species twice, and four species once only. By species, mean number and biomass where present ranged from 1 to 48.5 and from 0.001 to 11.145 kg per transect, respectively. Mean weight per individual ranged from 1 to 3184 g. By occurrence, the five most frequently encountered species were alewife, brown bullhead, yellow perch, pumpkinseed, and common carp. By numbers when present, the top five were alewife, yellow perch, brown bullhead, logperch, and emerald shiner. By biomass when present, the top five were common carp, common carp × goldfish hybrids, brown bullhead, bowfin, and lake trout.

In addition to fish catches, a variety of habitat data was obtained at subsets of transect locations with emphasis on submerged macrophyte occurrence and abundance (Table 2). In 1988, each of the 50 transects in Hamilton Harbour was assigned membership in one of four macrophyte density classes, absent (15 transects), sparse (7), moderate (10), and heavy (8), following a complete survey of macrophyte occurrence along the 1-m depth contour and confirmation that the categories corresponded to stem density groupings (V.W Cairns, unpublished data). In 1989, macrophyte biomass was estimated from duplicate quadrats and percent vegetation cover was diver-estimated along 33 electrofishing transects in the Bay of Quinte (Minns et al. 1993). In 1990, detailed surveys of macrophyte stem densities, composition, and percent cover were conducted by divers at 72 transects spread across the five areas surveyed (Minns et al. 1993).

Means for percent cover and stem density were substantially lower in Hamilton Harbour and the Bay of Quinte compared with the three areas in Severn Sound. The habitat subset of the electrofishing transects covered the full range of conditions present in the study areas.

## Methods

We developed an IBI for Great Lakes littoral fish assemblages, using the methods and philosophy laid out by Karr (1981, 1991), Karr et al. (1986), and Miller et al. (1988). Our method had five steps: (i) selection of IBI metrics, (ii) analysis of raw metrics, (iii) metric standardization and IBI formulation, (iv) analysis of IBI properties, and (v) analysis of relationships between IBI values and ecosystem and habitat conditions.

### Selection of IBI Metrics

To guide selection of IBI metrics, we reviewed those presented in published literature and reports (references marked with an asterisk). Metrics can be grouped in two ways: by the three categories used to group IBI metrics (species diversity, trophic composition, and abundance and condition) and by their mathematical formulation. Raw IBI metrics are always either the sum of a selected subset of species or individuals or the same sum expressed as a percentage of total for species presence–absence, abundance, or biomass. Metrics based on a subset of species richness and percentages of total abundance were the most frequent in the literature surveyed. Where IBIs have been developed for larger rivers and reservoirs, other forms of metrics, including some using biomass data, have been used. This review and consideration of the features of the littoral fish assemblages guided our selection of a set of IBI metrics.

### Analysis of Raw Metrics

We summarized the frequency of occurrences for the raw species richness metrics. For all metrics, we constructed cumulative percentage frequency tables using the whole database. We computed the Pearson correlations among all raw metrics and indices as a basis for examining redundancy among metrics. To assess the groupings and associations among metrics, we performed a principal components analysis using the selected, raw metrics as input.

### Metric Standardization and IBI Formulation

We adopted an approach to standardizing individual metrics and to the formation of an overall IBI similar to methods used by Barbolli et al. (1992). Karr (1981), and others, standardized metrics by assigning integer scores of 0, 1, 3, or 5 according to defined ranges and thresholds of each metric. The IBI is the sum of the integer scores with a maximum of 60 for the typical IBI with 12 metrics. Here, our object was to obtain an index in the form of a continuous variable, avoiding range gaps which arise when using integer scores with sequence gaps. We standardized each metric ensuring a minimum value of zero and a maximum of 10, using the following equation and conditions:

$$(1) \quad M_S = A + B \cdot M_R$$

$$\text{If } M_S < M_{\text{MIN}}, \text{ then } M_S = M_{\text{MIN}}$$

$$\text{If } M_S > M_{\text{MAX}}, \text{ then } M_S = M_{\text{MAX}}.$$

The equation defines a standardized metric ($M_S$) as linear function of a raw metric ($M_R$) (Fig. 2). The minimum and maximum thresholds ($M_{\text{MIN}}$ and $M_{\text{MAX}}$) define a floor and a ceiling for the standardized metric. A high value for the intercept, $A$, and a negative slope value, $B$, produces an inverse function; high values of some raw metrics imply low biotic integrity, denoted by low standardized metric values. This approach treats all raw metrics as continuous variables.

There are no objective rules for setting thresholds. As guidelines, we decided that (i) the floor should coincide with zero for positive metrics, (ii) the ceiling should begin near the 90–95th cumulative percentiles of the whole database, and (iii) the relationship between the raw and standardized metrics should be linear in between. For convenience and ease of explanation, we selected thresholds which

TABLE 1. List of fish species captured by electrofishing, 1988–90, in the three AOCs (BQ, Bay of Quinte; HH, Hamilton Harbour; Severn Sound: PH, Penetang Harbour; HB, Hog Bay; MB, Matchedash Bay), their frequency of occurrence by data set, and overall mean number and biomass (kg) per transect sample and mean weight per individual (g) where each species was encountered.

| | | Frequency of occurrence by data set | | | | | | | Mean when present | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Common name | Latin name | BQ89 | HH88 | BQ90 | HH90 | PH90 | HB90 | MB90 | Number | Biomass | Weight |
| Longnose gar | *Lepisosteus osseus* | 3 | 1 | 1 | 1 | — | — | — | 1.0 | 0.459 | 459 |
| Bowfin | *Amia calva* | 3 | 1 | 6 | — | 5 | 2 | 15 | 1.2 | 2.673 | 2227 |
| Alewife | *Alosa pseudoharengus* | 5 | 274 | 20 | 62 | 21 | 2 | 9 | 48.5 | 0.973 | 20 |
| Gizzard shad | *Dorosoma cepedianum* | 5 | 94 | 10 | 11 | — | — | 5 | 4.3 | 0.992 | 231 |
| Chinook salmon | *Oncorhynchus tshawytscha* | — | 32 | — | 5 | — | — | 1 | 1.6 | 0.016 | 10 |
| Rainbow trout | *Oncorhynchus mykiss* | — | — | — | 2 | — | — | — | 4.5 | 0.022 | 5 |
| Brown trout | *Salmo trutta* | — | 3 | — | 1 | — | — | — | 1.0 | 1.179 | 1179 |
| Lake trout | *Salvelinus namaycush* | — | 2 | — | 1 | — | — | — | 1.0 | 2.555 | 2555 |
| Rainbow smelt | *Osmerus mordax* | 2 | 4 | — | 7 | 2 | — | — | 2.3 | 0.022 | 10 |
| Northern pike | *Esox lucius* | 16 | 9 | 25 | 6 | 4 | 2 | 5 | 1.5 | 1.844 | 1229 |
| Central mudminnow | *Umbra limi* | 1 | — | 1 | — | — | — | — | 1.0 | 0.039 | 39 |
| Quillback | *Carpiodes cyprinus* | — | — | — | — | 12 | — | 1 | 1.5 | 1.515 | 1010 |
| White sucker | *Catostomus commersoni* | 10 | 59 | 9 | 16 | 39 | 3 | 22 | 2.2 | 1.325 | 602 |
| Silver redhorse | *Moxostoma anisurum* | — | — | 1 | — | 1 | — | — | 1.0 | 0.610 | 610 |
| Golden redhorse | *Moxostoma erythrurum* | — | — | — | — | — | — | 1 | 1.0 | 0.420 | 420 |
| Shorthead redhorse | *Moxostoma macrolepidotum* | 1 | — | 4 | — | 11 | — | — | 1.4 | 0.980 | 700 |
| Goldfish | *Carassius auratus* | — | 38 | — | 9 | 1 | — | 3 | 1.3 | 1.069 | 822 |
| Common carp | *Cyprinus carpio* | 13 | 188 | 19 | 37 | 11 | 5 | 8 | 3.5 | 11.145 | 3184 |
| Golden shiner | *Notemigonus crysoleucas* | 2 | — | 18 | — | 6 | 5 | 12 | 2.9 | 0.035 | 12 |
| Emerald shiner | *Notropis atherinoides* | 1 | 135 | 7 | 37 | 5 | — | — | 10.8 | 0.056 | 5 |
| Common shiner | *Luxilus cornutus* | 1 | — | — | — | — | — | — | 1.0 | 0.006 | 6 |
| Blackchin shiner | *Notropis heterodon* | — | — | — | — | 17 | 9 | — | 3.4 | 0.009 | 3 |
| Blacknose shiner | *Notropis heterolepis* | — | — | — | — | — | — | 1 | 1.0 | 0.003 | 3 |
| Spottail shiner | *Notropis hudsonius* | 19 | 67 | 19 | 2 | 6 | — | 4 | 4.0 | 0.020 | 5 |
| Bluntnose minnow | *Pimephales notatus* | — | — | — | — | 15 | 3 | 1 | 3.8 | 0.007 | 2 |
| Carp × goldfish | Hybrid | — | 3 | — | — | — | — | — | 2.0 | 3.830 | 1915 |
| Brown bullhead | *Ameiurus nebulosus* | 9 | 215 | 18 | 42 | 18 | 2 | 24 | 19.4 | 3.586 | 185 |
| Channel catfish | *Ictalurus punctatus* | 1 | — | — | 1 | — | — | — | 1.0 | 1.095 | 1095 |
| American eel | *Anguilla rostrata* | 10 | 15 | 7 | 5 | — | — | — | 1.1 | 1.117 | 1015 |
| Banded killifish | *Fundulus diaphanus* | — | — | — | — | 1 | — | — | 1.0 | 0.001 | 1 |
| Trout-perch | *Percopsis omiscomaycus* | — | 1 | — | — | 1 | — | — | 3.5 | 0.036 | 10 |
| White perch | *Morone americana* | 17 | 163 | 13 | 38 | — | — | — | 10.1 | 0.816 | 81 |
| White bass | *Morone chrysops* | 1 | 22 | 2 | 2 | 2 | — | 4 | 2.0 | 0.493 | 246 |
| Rock bass | *Ambloplites rupestris* | 24 | 3 | 25 | 2 | 26 | 9 | 27 | 3.3 | 0.262 | 79 |
| Pumpkinseed | *Lepomis gibbosus* | 26 | 102 | 31 | 20 | 62 | 25 | 31 | 8.8 | 0.387 | 44 |
| Bluegill | *Lepomis macrochirus* | 1 | — | 9 | 1 | — | — | — | 3.2 | 0.183 | 57 |
| Smallmouth bass | *Micropterus dolomieu* | 14 | 23 | 15 | 5 | 34 | 2 | 1 | 2.5 | 0.543 | 217 |
| Largemouth bass | *Micropterus salmoides* | 8 | 17 | 13 | 9 | 30 | 20 | 18 | 2.9 | 0.648 | 223 |
| Black crappie | *Pomoxis nigromaculatus* | 2 | 3 | 7 | 2 | 23 | 15 | 25 | 3.2 | 0.138 | 43 |
| Yellow perch | *Perca flavescens* | 26 | 118 | 47 | 5 | 69 | 25 | 35 | 21.7 | 0.699 | 32 |
| Walleye | *Stizostedion vitreum* | 27 | — | 27 | — | 1 | 1 | 5 | 4.4 | 1.429 | 325 |
| Johnny darter | *Etheostoma nigrum* | 1 | 1 | 2 | — | — | — | — | 1.3 | 0.005 | 4 |
| Logperch | *Percina caprodes* | 20 | — | 20 | — | — | — | — | 11.8 | 0.082 | 7 |
| Brook silverside | *Labidesthes sicculus* | — | — | 6 | — | 4 | 3 | — | 1.2 | 0.002 | 2 |

Table 1. (*Concluded*)

| Common name | Latin name | Frequency of occurrence by data set | | | | | | | Mean when present | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BQ89 | HH88 | BQ90 | HH90 | PH90 | HB90 | MB90 | Number | Biomass | Weight |
| Freshwater drum | *Aplodinotus grunniens* | 19 | 17 | 15 | 5 | — | — | — | 1.8 | 1.670 | 928 |
| No. of transects | | 32 | 40 | 20 | 48 | 29 | 14 | 12 | | | |
| No. of samples | | 34 | 326 | 59 | 85 | 84 | 28 | 38 | | | |
| Samples/transect | | 1–2 | 7–11 | 1–3 | 2–3 | 2–3 | 2 | 3 | | | |
| No. of species | | 30 | 28 | 29 | 27 | 27 | 17 | 24 | | | |

were round numbers. Zero is a logical floor for positive metrics, as the absence of fish is the worst possible case. The 90–95th percentiles as the ceiling for metrics were chosen for two reasons. First, the maximum value is a rare occurrence and hence difficult to attain. Second, an analogy was drawn with usual probability levels for statistical significance. For positive metrics the values at the 90–95th percentiles are assumed to be a measure of the levels attainable for any transect not subject to degradation or disturbance. The linear relationship between the thresholds is the simplest model available.

The standardization step used in this study is based on the assumption that impairment of beneficial ecosystem uses is never a universal phenomenon within an AOC. Further, we have assumed that a survey database drawn from three AOCs with a directed effort to sample the widest range of habitat types will include samples that represent acceptable reference bench marks. Given the extent of ecosystem modifications along the shorelines of the Great Lakes, and their concentration in bays, true "control" sites for AOCs are unlikely to exist.

Next, standardized metrics are summed and multiplied by $10/N_M$, where $N_M$ is the number of metrics, to produce an IBI with a minimum of 0 and a maximum of 100. A value of 0 is only obtained when the sampling effort produces no catch. Catches of one or a few fish from one species will produce IBI values >0.

Finally, qualitative IBI categories were named for five equal-width IBI ranges for those sample transects where fish were captured: 0 = No Fish, >0–20 = Very Poor, >20–40 = Poor, >40–60 = Fair, >60–80 = Good, and >80 = Excellent. Karr (1981) and others have used similar categories to provide a qualitative interpretation for nontechnical audiences.

### Analysis of IBI Properties

Angermeier and Karr (1986) indicated that individual metrics do not contribute uniformly to IBI values over the whole IBI range but did not offer any specific evidence or analysis. The relative sensitivity of IBI values to individual metrics was examined among metrics and among IBI categories for each metric. We assessed IBI sensitivity to each metric by computing a reduced IBI using the other metrics (Reduced IBI = $10\cdot(N_M\cdot\text{IBI}/10 - \text{Test Metric})/(N_M - 1)$) and computing the difference between reduced and overall IBIs. The variances of differences give an indication of the relative importance of individual metrics. For each metric, the ratio of the variance of differences within an IBI range category (Excellent, Good, etc.) to the total variance of differences provides a measure of the range sensitivity: a metric with a high ratio affects IBI values in a particular category more than one with a low ratio.

The overall distribution of IBI values was assessed for normality using a Kolmogorov–Smirnov (K–S) $D$ statistic and alternative transformations evaluated for their ability to produce a normal distribution. IBI values were transformed before subsequent statistical analyses were performed. The variability of IBI values was assessed in three ways: (i) calculation of seasonal means and standard errors where the number of samples at a transect site in a year was three or greater, (ii) calculation of the mean and standard deviation of absolute IBI differences between pairs of samples collected at transects within a few days of each other,

TABLE 2. Sample size, mean, minimum, and maximum of percent cover, plant biomass (in 1989), and stem density (in 1990) data collected on a subset of the electrofishing transects surveyed in 1989 and 1990.

| | Percent cover | | | | Biomass (g) or stem density (no./m$^2$) | | | |
|---|---|---|---|---|---|---|---|---|
| Area and year | *N* | Mean | Min. | Max. | *N* | Mean | Min. | Max. |
| 1989 | | | | | | | | |
| Bay of Quinte | 33 | 38.9 | 0.0 | 100.0 | 33 | 39.0 | 0.0 | 288.5 |
| 1990 | | | | | | | | |
| Bay of Quinte | 12 | 21.6 | 0.0 | 87.0 | 13 | 22.4 | 0.0 | 105.0 |
| Hamilton Harbour | 20 | 11.2 | 0.5 | 79.0 | 20 | 17.3 | 0.0 | 170.1 |
| Severn Sound | | | | | | | | |
| Penetang Harbour | 16 | 65.5 | 5.0 | 100.0 | 18 | 1303.5 | 79.9 | 7126.8 |
| Hog Bay | 10 | 57.9 | 5.0 | 99.0 | 10 | 216.7 | 47.4 | 724.7 |
| Matchedash Bay | 10 | 76.1 | 5.0 | 100.0 | 11 | 545.0 | 7.8 | 3145.4 |

TABLE 3. Codes and descriptions for 12 metrics selected for the Great Lakes littoral fish assemblage IBI with the coefficients intercept (A) and slope (*B*) in equation 1 for standardizing the metrics and the values of each raw metric ($M_R$) which define the limits of the standardized metric ($M_S$) as shown in Fig. 2.

| | | Metric coefficients | | Values of raw metric ($M_R$) where | |
|---|---|---|---|---|---|
| Code | Metric description | *A* | *B* | $M_S = 0$ | $M_S = 10$ |
| Species richness | | | | | |
| SNAT | Natives | 0 | 10/8 | 0 | ≥8 |
| SCEN | Centrarchids | 0 | 10/3 | 0 | ≥3 |
| SINT | Intolerants | 0 | 5 | | ≥2 |
| SNIN | Nonindigenous | 10 | −10/3 | ≥3 | 0 |
| SCYP | Native cyprinids | 0 | 5 | 0 | ≥2 |
| Trophic structure | | | | | |
| PPIS | % piscivore biomass | 0 | 0.3 | 0.0 | ≥33.3 |
| PGEN | % generalist biomass | 15 | −0.15 | 100.0 | ≥33.3 |
| PSPE | % specialist biomass | 0 | 0.3 | 0 | ≥33.3 |
| Abundance and condition | | | | | |
| NNAT | No. of native individuals | 0 | 1/12 | 0 | ≥120 |
| BNAT | Biomass of natives (kg) | 0 | 5/6 | 0 | ≥12 |
| PNNI | % nonindigenous numbers | 10 | −0.1 | 100 | 0 |
| PBNI | % nonindigenous biomass | 10 | −0.1 | 100 | 0 |

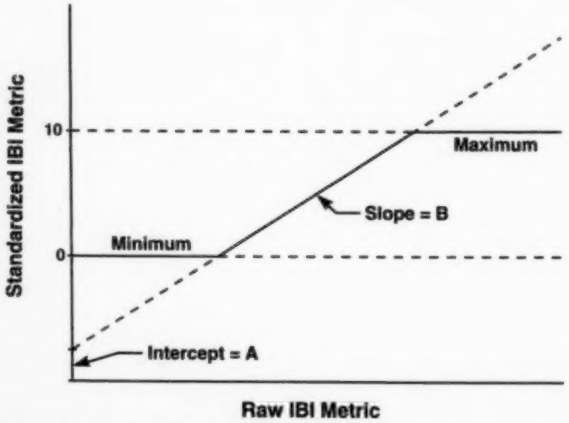

FIG. 2. Algebraic rules for producing a standardized IBI metric from a raw metric (see text for further explanation).

and (iii) calculation of Pearson correlations of transect mean values between years in the Bay of Quinte (1989 versus 1990) and Hamilton Harbour (1988 versus 1990).

### Analysis of Relationships between IBI Values and Ecosystem and Habitat Conditions

IBI values were evaluated at two levels: between ecosystems according to general conditions and, within ecosystems, among transects in relation to site-specific habit features. First, the seven groups of transect means were assigned as treatments in a one-way analysis of variance. Second, a series of regression analyses and analyses of covariance were performed to relate transect means to measures of vegetation abundance. To examine relationships between IBI values and habitat features, we used both the IBI itself and a modified form, IBI*, adjusted for the mean of the proportions of fish numbers ($P_{\mathrm{N.OFF}}$) and biomass ($P_{\mathrm{B.OFF}}$) in samples attributable to incursions of "offshore" fish species:

TABLE 4. Group membership assignments, for all fish species captured in the three AOCs, used to define all 12 metrics listed in Table 3. Cen, centrarchids; Cyp, native cyprinids; Pis; piscivore; Gen, generalist; Spe, specialist.

| Common name | Native? | Taxa | Turbidity tolerant? | Trophic group | Offshore? |
|---|---|---|---|---|---|
| Longnose gar | Y | | | Pis | |
| Bowfin | Y | | | Pis | |
| Alewife | N | | | Spe | Y |
| Gizzard shad | Y | | | Spe | Y |
| Chinook salmon | N | | | Pis | Y |
| Rainbow trout | N | | | Spe | Y |
| Brown trout | N | | | Pis | Y |
| Lake trout | Y | | | Pis | Y |
| Rainbow smelt | N | | | Spe | Y |
| Northern pike | Y | | | Pis | |
| Central mudminnow | Y | | | Gen | |
| Quillback | Y | | | Gen | |
| White sucker | Y | | | Spe | |
| Silver redhorse | Y | | | Spe | |
| Golden redhorse | Y | | | Spe | |
| Shorthead redhorse | Y | | | Spe | |
| Goldfish | N | | | Gen | |
| Common carp | N | | | Gen | |
| Golden shiner | Y | Cyp | | Gen | |
| Emerald shiner | Y | Cyp | | Spe | |
| Common shiner | Y | Cyp | | Spe | |
| Blackchin shiner | Y | Cyp | | Spe | |
| Blacknose shiner | Y | Cyp | N | Spe | |
| Spottail shiner | Y | Cyp | N | Spe | |
| Bluntnose minnow | Y | Cyp | | Gen | |
| Carp × goldfish | N | | | Gen | |
| Brown bullhead | Y | | | Gen | |
| Channel catfish | Y | | | Gen | |
| American eel | Y | | | Pis | |
| Banded killifish | Y | | | Spe | |
| Trout-perch | Y | | | Spe | |
| White perch | N | | | Spe | Y |
| White bass | Y | | | Spe | Y |
| Rock bass | Y | Cen | N | Spe | |
| Pumpkinseed | Y | Cen | | Spe | |
| Bluegill | Y | Cen | | Spe | |
| Smallmouth bass | Y | Cen | | Pis | |
| Largemouth bass | Y | Cen | | Pis | |
| Black crappie | Y | Cen | | Spe | |
| Yellow perch | Y | | | Spe | |
| Walleye | Y | | | Pis | Y |
| Johnny darter | Y | | | Spe | Y |
| Logperch | Y | | | Spe | Y |
| Brook silverside | Y | | N | Spe | |
| Freshwater drum | Y | | | Spe | |

$$IBI^* = IBI \cdot \{1 - P_{OFF}\}$$

$$P_{OFF} = \{P_{N.OFF} + P_{B.OFF}\}/2.$$

Simple sample population statistics were computed using features of the RS/1 data analysis program (BBN Software Products Corporation, Cambridge, Mass.). More complicated statistical procedures were performed using the PC version 5.0 of Systat (Wilkinson 1990).

## Results

### Selection of IBI Metrics

We selected 12 metrics for inclusion in the IBI (Table 3) and assigned species to metrics using group memberships (Table 4). Metrics related to disease or anomalies were not considered, as the necessary data were not collected. Metrics of total species richness, abundance, or biomass were not used, as they are sums of assemblage components which measure opposite features of integrity, e.g., native versus nonindigenous species.

Five species richness metrics included natives (SNAT), centrarchids (SCEN), native cyprinids (SCYP), nonindigenous species (SNIN), and "intolerant" species (SINT), those species reported to be intolerant of increased turbidity. Native species richness is a strong overall indicator of ecosystem health. In the Great Lakes, nonindigenous species have had a major impact on fish assemblages (Emery 1985); SNAT is a positive indicator, while SNIN is a negative one.

TABLE 5. Percent frequency of occurrence for fish species richness values in six taxonomic categories based on 654 transect samples from surveys in three Great Lakes' AOCs.

| Species richness | Total (STOT) | Natives (SNAT) | Centrarchids (SCEN) | Nonindigenous (SNIN) | Native cyprinids (SCYP) | Turbidity intolerants (SINT) |
|---|---|---|---|---|---|---|
| 0 | 2.0 | 7.3 | 44.6 | 22.0 | 50.9 | 68.7 |
| 1 | 5.2 | 12.5 | 24.3 | 26.9 | 39.1 | 25.4 |
| 2 | 8.4 | 17.6 | 15.1 | 30.0 | 9.0 | 5.5 |
| 3 | 11.6 | 14.5 | 10.2 | 15.7 | 0.9 | 0.5 |
| 4 | 14.1 | 14.7 | 5.0 | 5.2 | | |
| 5 | 12.4 | 10.1 | 0.6 | 0.2 | | |
| 6 | 15.4 | 9.3 | | | | |
| 7 | 11.2 | 5.7 | | | | |
| 8 | 8.1 | 2.9 | | | | |
| 9 | 5.0 | 2.4 | | | | |
| 10 | 3.5 | 2.0 | | | | |
| 11 | 1.5 | 0.2 | | | | |
| 12 | 0.8 | 0.8 | | | | |
| 13 | 0.3 | | | | | |
| 14 | 0.5 | | | | | |

SCEN and SCYP species richnesses are positive metrics which have been widely used in lotic IBIs. These two groups are typical of temperate North American littoral areas and greater richness is expected when habitat conditions are undisturbed. The presence of species intolerant of elevated turbidity has been a prominent metric in many lotic IBIs (Fausch et al. 1990; Karr 1911). As turbidity is often cited as an important fish habitat factor in littoral areas on the Great Lakes, we used references to requirements for clearer waters in Scott and Crossman (1973) along with designation lists included in published IBI papers to identify four turbidity-intolerant species in the littoral fish assemblages (Table 4). The concept of "intolerance" need not be restricted to turbidity but might include any man-made impact. A narrow definition has been used in this study.

Trophic structure metrics were based on proportions of total biomass, and fish species were assigned to one of three groups: piscivores, generalists, and specialists (Table 4). Generalists include species noted for their multitrophic, highly adaptable diets. Specialists are those herbivores, planktivores, insectivores, arthropodivores, etc., and their proportional biomass is considered a positive fish assemblage indicator. Piscivores exert a top-down regulation of fish and lower trophic level assemblages. Low piscivore contribution to abundance is evidence of poor conditions. Using Scott and Crossman (1973) and Portt et al. (1988) as primary sources if guidance, we classified species as piscivores if their non-YOY diet consisted predominantly of fish, as generalists if their diet spectrum was judged to be wide, and as specialists if they did not appear to fit the other two categories.

Abundance and condition metrics were based on native numbers and biomass (NNAT and BNAT), nonindigenous species as a percentage of total number and biomass (PNNI and PBNI). Metrics based on both numbers and biomass were used because of the wide variation of fish sizes among the species encountered in littoral transects, a situation of less concern in the development of small-stream IBIs. Numbers and biomass of native species were used instead of totals because of the substantial influence of nonindigenous species, particularly alewife on numbers and carp on biomass. PNNI and PBNI measure the impact of nonindegenous species on total catches.

### Analysis of Raw Metrics

Total and native species richness values (STOT and SNAT) per transect sample had similar ranges: 0–12 versus 0–14 (Table 5). The most frequent values of STOT and SNAT were 6 and 2 at 15.4 and 17.6%, respectively. For STOT, 53.7% of values were <6 and 93.4% <10. Samples from the Bay of Quinte and Penetang Harbour accounted for most STOT values >9. For SNAT, 52.0% of values were <4 and 91.7% <8. SNAT values in Hamilton Harbour were much lower than those in other areas.

The species richness values for centrarchid (SCEN) and nonindigenous (SNIN) fish ranged from 0 to 5, while those of turbidity intolerants (SINT) and native cyprinids (SCYP) ranged from 0 to 3 (Table 5). The percent frequency values for these richness metrics varied much more than for STOT and SNAT. SCEN values tended to be higher in Severn Sound and lower in Hamilton Harbour, while the opposite trend was evident for SNIN values. Intolerants were uncommon in Hamilton Harbour; a single species was encountered in 74 of 411 samples. They were more common in the Bay of Quinte; two or three species were encountered in 26 of 93 samples and one or more species in 65 samples. The frequencies of SCYP values were similar in all areas. Centrarchids were absent from 44.7% of samples, intolerants from 68.7%, and native cyprinids from 50.9%, but nonindigenous species were only absent from 22.0%; species indicative of healthy fish assemblages were less likely to be present. In SNIN values, 49% of samples had values <2 and 95% <4. SNIN values were lowest in Severn Sound and highest in Hamilton Harbour.

Frequency distributions of the trophic composition metrics, percentages by biomass of piscivores (PPIS), generalists (PGEN), and specialists (PSPE), showed that piscivores made up a small part of transect catches, while proportions of generalists and specialists were more evenly represented across the range of values (Table 6C). Mean percentages of PPIS, PGEN, and PSPE were 11.1, 42.1, and 44.9, respectively. Percent piscivore biomass was <10 in 73.2% of samples, <40 in 88.8%, and <50 in 91.9%.

The abundance and biomass of native species (NNAT and BNAT) ranged 0–539 and 0–33.3 kg, respectively. While the maximum of NNAT closely approached that for maximum

TABLE 6. Cumulative percent distributions of (A) numbers and biomass of native species caught per transect sample, (B) percent contribution of nonindigenous species to total numbers and biomass, and (C) percent trophic composition of total biomass by transect sample based on 654 electrofishing samples in seven data sets.

| (A) Catch of native species | | | | (B) Percent nonindigenous in catch | | | (C) Trophic composition by biomass | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number | | Biomass | | | Cumulative percentage | | | Cumulative percentage | | |
| Range | Cumulative percentage | Range (kg) | Cumulative percentage | Range | Number | Biomass | Range | Piscivores | Generalists | Specialists |
| 0 to <20 | 57.0 | 0 to <2 | 47.7 | 0 to <10 | 36.4 | 33.8 | 0 to <10 | 73.2 | 39.1 | 24.6 |
| 20 to <40 | 69.9 | 2 to <4 | 69.0 | 10 to <20 | 42.1 | 38.5 | 10 to <20 | 79.8 | 44.0 | 37.2 |
| 40 to <60 | 80.1 | 4 to <6 | 79.7 | 20 to <30 | 46.5 | 41.1 | 20 to <30 | 84.4 | 47.3 | 46.2 |
| 60 to <80 | 87.0 | 6 to <8 | 85.6 | 30 to <40 | 50.5 | 46.5 | 30 to <40 | 88.8 | 52.1 | 53.1 |
| 80 to <100 | 91.9 | 8 to <10 | 89.8 | 40 to <50 | 55.2 | 52.1 | 40 to <50 | 91.9 | 55.4 | 59.0 |
| 100 to <120 | 94.5 | 10 to <12 | 92.5 | 50 to <60 | 60.2 | 58.1 | 50 to <60 | 95.4 | 59.9 | 63.5 |
| 120 to <140 | 97.1 | 12 to <14 | 94.6 | 60 to <70 | 65.6 | 64.7 | 60 to <70 | 97.7 | 64.8 | 68.5 |
| 140 to <160 | 97.9 | 14 to <16 | 96.8 | 70 to <80 | 72.2 | 70.3 | 70 to <80 | 98.2 | 71.9 | 72.9 |
| 160 to <180 | 98.6 | 16 to <18 | 97.6 | 80 to <90 | 80.4 | 80.9 | 80 to <90 | 99.1 | 81.2 | 78.6 |
| 180 to <200 | 98.9 | 18 to <20 | 98.2 | 90 to <100 | 94.7 | 94.7 | 90 to 100 | 100.0 | 100.0 | 100.0 |
| 200 to <220 | 99.2 | 20 to <22 | 98.6 | 100 | 100.0 | 100.0 | | | | |
| ≥220 | 100.0 | ≥22 | 100.0 | | | | | | | |

TABLE 7. Matrix of Pearson correlations ($r$) among 12 raw, unstandardized IBI metrics and with three raw total metrics (STOT, total species richness; NTOT, total catch; and BTOT, total biomass per sample), percent offshore contribution ($P_{OFF}$), and the computed IBI* and IBI indices; Bonferroni-adjusted probabilities for these values indicate that $r \geq 0.15$ is significant at nominal $P = 0.05$ and $r \geq 0.17$ (bold) is significant at nominal $P = 0.005$ on 653 df.

| | SCEN | SINT | SNIN | SCYP | PPIS | PGEN | PSPE | NNAT | BNAT | PNNI | PBNI | STOT | NTOT | BTOT | $P_{OFF}$ | IBI* | IBI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SNAT | **0.79** | **0.68** | −0.11 | **0.45** | **0.40** | −0.13 | −0.00 | **0.54** | **0.34** | **−0.48** | **−0.39** | **0.90** | **0.25** | 0.10 | **−0.34** | **0.73** | **0.81** |
| SCEN | | **0.55** | **−0.30** | **0.17** | **0.38** | **−0.20** | 0.05 | **0.48** | **0.23** | **−0.53** | **−0.43** | **0.62** | 0.14 | −0.01 | **−0.45** | **0.77** | **0.76** |
| SINT | | | −0.12 | **0.44** | **0.29** | −0.10 | −0.02 | **0.38** | 0.16 | **−0.29** | **−0.21** | **0.59** | **0.21** | 0.05 | **−0.19** | **0.50** | **0.59** |
| SNIN | | | | 0.14 | **−0.29** | **0.48** | **−0.28** | −0.03 | 0.12 | **0.57** | **0.62** | **0.33** | **0.35** | **0.48** | **0.41** | **−0.53** | **−0.46** |
| SCYP | | | | | 0.01 | −0.00 | 0.04 | **0.32** | 0.08 | −0.03 | 0.04 | **0.49** | **0.31** | 0.13 | 0.01 | **0.22** | **0.33** |
| PPIS | | | | | | **−0.36** | −0.14 | 0.11 | 0.13 | **−0.35** | **−0.42** | **0.25** | −0.10 | −0.14 | **−0.20** | **0.46** | **0.54** |
| PGEN | | | | | | | **−0.80** | 0.03 | **0.24** | **0.20** | **0.48** | 0.08 | 0.06 | **0.60** | **−0.24** | **−0.25** | **−0.47** |
| PSPE | | | | | | | | −0.06 | **−0.29** | 0.04 | **−0.21** | −0.12 | 0.05 | **−0.51** | **0.43** | 0.07 | **0.30** |
| NNAT | | | | | | | | | **0.61** | **−0.42** | **−0.28** | **0.50** | **0.63** | **0.30** | **−0.33** | **0.54** | **0.53** |
| BNAT | | | | | | | | | | **−0.34** | **−0.29** | **0.38** | **0.40** | **0.55** | **−0.30** | **0.36** | **0.34** |
| PNNI | | | | | | | | | | | **0.78** | **−0.21** | **0.19** | 0.10 | **0.76** | **−0.81** | **−0.67** |
| PBNI | | | | | | | | | | | | −0.11 | 0.15 | **0.32** | **0.48** | **−0.70** | **−0.70** |

TABLE 8. Results of PCA for the 12 raw, unscaled metrics used in the IBI after selection of components with eigenvalues >1 and varimax rotation. The highest component coefficient for each metric is underlined to show to which component the varimax rotation maximized its weighting.

| Variable | Principal components 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Eigenvalue | 4.249 | 2.467 | 1.542 | 1.056 |
| Percentage of variance explained | | | | |
| Before rotation | 35.407 | 20.558 | 12.846 | 8.803 |
| After rotation | 22.316 | 15.941 | 23.860 | 15.498 |
| Cumulative | 22.316 | 38.257 | 62.117 | 77.615 |
| Coefficients | | | | |
| SINT | 0.838 | 0.024 | 0.148 | 0.048 |
| SNAT | 0.833 | 0.012 | 0.284 | 0.291 |
| SCYP | 0.698 | −0.151 | −0.337 | 0.139 |
| SCEN | 0.651 | −0.022 | 0.460 | 0.228 |
| PSPE | −0.004 | −0.969 | 0.007 | −0.072 |
| PGEN | −0.115 | 0.804 | −0.422 | 0.201 |
| PBNI | −0.080 | 0.266 | −0.802 | −0.339 |
| SNIN | 0.054 | 0.334 | −0.762 | 0.048 |
| PNNI | −0.176 | 0.019 | −0.756 | −0.440 |
| PPIS | 0.373 | 0.197 | 0.668 | −0.226 |
| BNAT | 0.091 | 0.283 | 0.106 | 0.823 |
| NNAT | 0.413 | −0.015 | 0.071 | 0.785 |

total abundance, 541, the maximum from BNAT was less than half that of total biomass, 71.9 kg, due to the great influence of carp occurrences. The distributions of NNAT and BNAT were heavily weighted to lower values with 57.0% of abundances in the 0–20 range and 47.7% of biomasses in the 0–2 kg range (Table 6A). The frequency distributions were Poisson-like. In contrast with those of NNAT and BNAT, the distributions of percent nonindigenous abundance and biomass (PNNI and PBNI) were more even (Table 6B) although the 0 to <10% range accounted for 36.4 and 33.8% of the occurrences of PNNI and PBNI, respectively, and nearly 20% of cases were 80% or greater.

Many of the correlations among raw metrics and with other variables were significant, at Bonferroni-adjusted probabilities equivalent to a nominal significance level $P = 0.05$ (Table 7). Among the IBI metrics, native species richness (SNAT) had significant, positive correlations with three other species richness metrics (SNIN was the exception), with percent piscivore biomass (PPIS) and native abundance (NNAT) and biomass (BNAT) and significant negative correlations with the percent nonindigenous metrics (PNNI and PBNI). Centrarchids (SCEN) and turbidity intolerants (SINT) showed similar patterns of significant correlations. Native cyprinid richness (SCYP) was not significantly correlated with PPIS, BNAT, PNNI, or PBNI. Percent piscivore biomass (PPIS) had significant negative correlations with percent generalist biomass (PGEN) as well as with PNNI and PBNI. PGEN was negatively correlated with percent specialist biomass (PSPE) but positively correlated with BNAT, PNNI, and PBNI. NNAT and BNAT were positively correlated and both were negatively correlated with PNNI and PBNI which were positively correlated.

Many correlations of the 12 raw metrics with metrics of total richness (STOT), abundance (NTOT), and biomass (BTOT), percent offshore ($P_{OFF}$), and the IBIs, before and after multiplication by $(1 - P_{OFF})$, were significant (Table 7). Total species richness was significantly correlated with 9 of 12 metrics. One of the nine was negative. Total abundance was positively correlated with seven metrics and total biomass was correlated with six metrics; that with PSPE was negative. Total abundance was not significantly correlated with the trophic composition metrics and total biomass was not with four of five species richness metrics. Total biomass was positively correlated with nonindigenous species richness due to the large contribution of introduced common carp. The composite offshore metric, $P_{OFF}$, was negatively correlated with 7 of 12 metrics. The IBI was correlated, as expected, with all 12 metrics and the sign corresponded to the positive or negative formulations used in standardizing the metrics. The $P_{OFF}$-adjusted IBI* was significantly correlated with all metrics but PSPE.

Principal component analysis (PCA) of the 12 raw, unstandardized metrics produced the result that four components with eigenvalues >1 accounted for 77.6% of the variance (Table 8). After varimax rotation, the loadings for the metrics were grouped to reveal four main features of the data set. Component 1 indicated species richness with four of five species richness metrics loading positively. Component 2 showed a contrast between the proportions of total biomass designated as generalist (PGEN) and specialist (PSPE). Component 3 showed another contrast between the proportion of total biomass as piscivores (PPIS) and three metrics measuring the influence of nonindigenous species (SNIN, PNNI, and PBNI). Finally, component 4 was an indicator of abundance/biomass for native species (NNAT and BNAT). The sign and weight of the dominant loadings for each metric were consistent with the metric's expected role in the IBI.

### Metric Standardization and IBI Formulation

The values of the standardization coefficients for the metrics are shown in Table 3 and the rationale for setting the intercepts and slopes follows.

For the species richness metrics, the levels defining the maximum for all five were placed close to the 95th percentiles in the cumulative frequencies of occurrence. In native species (SNAT), zero to eight species accounted for 94.7% of the data set (Table 5) and the maximum was set at eight species, i.e., eight or more species produced a metric value of 10. In centrarchid (SCEN) and nonindigenous (SNIN) species, zero to three species accounted for 94.3 and 94.7% of the data set, respectively (Table 5). For SCEN, a positive maximum was set at three, while for SNIN, a negative metric was used with three or more species producing a metric value of 0. For intolerant species (SINT), zero and one species accounted for 94.0% and zero to two species accounted for 99.6% of the data set. To avoid having a binary metric, the metric was set so that two species or more produced a metric value of 10. Using one or more binary metrics would create the possibility of dividing the resultant IBI indices into discrete groups with those metrics having excessive influence on the outcome. For native cyprinids (SCYP), zero and one species accounted for 90.1%, while zero to two accounted for 99.1% of the data set. Again, the SCYP species value was set at two to produce a metric value of 10.

For the trophic structure metrics, the 95th percentiles did not appear to be a useful guideline for standardizing the

TABLE 9. Ratios of within-class variance to total variance of IBI values with and without each metric, showing the sensitivity of IBI values to the addition of each metric for different IBI classes, i.e., different ranges of IBI values (underlined values are greater than the median ratio in the set, indicating IBI classes where individual metrics make a greater contribution).

| | IBI class | | | | | |
|---|---|---|---|---|---|---|
| Metric | Excellent | Good | Fair | Poor | Very Poor | Total variance |
| SNAT | 0.12 | 0.78 | 1.56 | 0.94 | 0.59 | 2.38 |
| SCEN | 0.46 | 0.88 | 1.45 | 0.72 | 0.30 | 5.16 |
| SINT | 1.45 | 1.65 | 1.25 | 0.56 | 0.22 | 4.83 |
| SNIN | 0.60 | 0.76 | 1.46 | 0.86 | 0.80 | 6.72 |
| SCYP | 0.75 | 1.02 | 1.27 | 0.74 | 0.40 | 9.17 |
| PPIS | 1.20 | 1.84 | 1.76 | 0.38 | 0.04 | 7.14 |
| PGEN | 0.02 | 0.16 | 0.97 | 1.55 | 0.73 | 8.49 |
| PSPE | 0.39 | 0.38 | 0.96 | 1.33 | 0.83 | 9.45 |
| NNAT | 0.86 | 1.35 | 1.36 | 0.85 | 0.07 | 4.94 |
| BNAT | 0.78 | 0.60 | 1.24 | 1.00 | 0.08 | 6.09 |
| PNNI | 0.02 | 0.17 | 1.17 | 1.22 | 0.48 | 5.85 |
| PBNI | 0.14 | 0.45 | 1.22 | 1.07 | 0.09 | 5.32 |

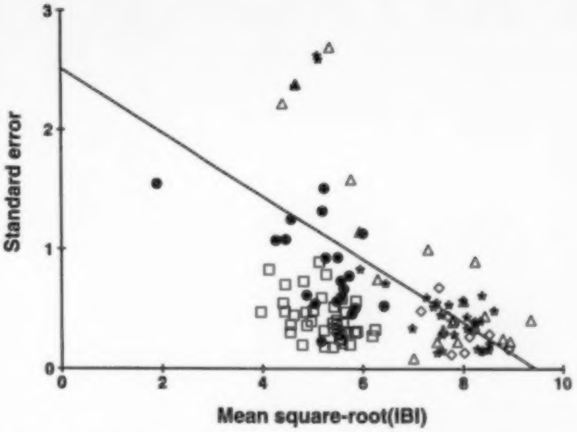

FIG. 3. Standard error versus mean of $\sqrt{\mathrm{IBI}}$ values, where sample size was ≥3 within one year, from five data sets: Hamilton Harbour in 1988 (□) and 1990 (●), Bay of Quinte in 1990 (△), Penetang Harbour in 1990 (*), and Matchedash Bay in 1990 (◇). The line shows predicted values of standard error given mean $\sqrt{\mathrm{IBI}}$ with $N = 3$, using the regression model described in the text.

metrics and a modified approach was adopted. Higher percentages of piscivore and specialist biomass are positive indicators of biotic integrity, while higher percentages of generalists, largely consisting of carp, goldfish, their hybrids, and ictalurids, are a negative indicator. This view of trophic composition is consistent with Pimm's (1982) idea that species feeding at several trophic levels (generalists) tend to destabilize assemblages, while species at the top of the food web (piscivores) impose a top-down regulation of assemblage composition. This view is also consistent with Kerr's (1977) hypothesis that a more even distribution of biomass across the size spectrum, and hence across trophic groupings, is characteristic of less disturbed and more "balanced" aquatic ecosystems. The 95th percentile for piscivores occurred in the 50–60% of biomass range, for generalists in the 90–100% range, and for specialists in the 90–100% range. Setting the PPIS maximum at 50% for piscivores while setting the minimum for PGEN at 100% for generalists and the maximum for PSPE at 100% would have meant that the maximum combined metric score for trophic structure was 20. Since the three metrics were percentages of total biomass summing to 100, it seemed reasonable that the standardization equations should be complementary, i.e., the combined range of the metrics should be 0–30. Setting the PPIS and PSPE maxima and the PGEN minimum at 33.3% of total biomass ensured a maximum score of 30 for the sum of the three metrics, this occurs if the biomass is equally divided among the three trophic groups.

In the abundance and condition group of metrics, the 90–95th percentiles guideline was the basis for selecting the maximum threshold for numbers and biomass of native species, NNAT and BNAT. All biotic assemblages are parts of successional sequences which characteristically accumulate species and abundance (Odum 1969). For NNAT, the range 0 to <120 accounted for 94.5% of values (Table 6A) and the maximum was set at 120. For BNAT, the range 0 to <12 kg accounted for 92.5% of values, increasing to 94.6% for <14 and to 97.6% for <16 kg. We selected 12 kg as the threshold for BNAT. For the percent contributions of nonindigenous species to total abundance and biomass (PNNI and PBNI), we matched the standardized metric range to the full range of percentages. Although large proportions of the samples lay in the 0–10 and 80–100% ranges, cumulative percent frequencies of both PNNI and PBNI increased almost linearly over the 10–90% range.

The 12 standardized metrics were summed and multiplied by 10/12 to produce the final IBI with a potential range of 0–100. Eight of the metrics are based on positive weighting and four on negative. Three of the negative ones directly relate to nonindigenous metrics for species richness, abundance, and biomass, and the fourth, percent generalist biomass, indirectly relates to nonindigenous species, as common carp often accounted for much of the generalist biomass.

### Sensitivity of IBI to Individual Metrics

Total variances of differences between overall and reduced IBI values, obtained by removing each metric, ranged from 2.38 for native species richness (SNAT) to 9.45 for percent specialist biomass (PSPE) with a mean of 6.30 (Table 9). Between metrics, the total variances are indicative of the relative sensitivity of the overall IBI values to individual metrics. One species richness (SCYP) and two trophic composition metrics (PGEN and PSPE) had the highest variances. The three lowest variances were due to two richness metrics (SNAT and SINT) and an abundance metric (NNAT).

The sensitivities of the named IBI classes to individual metrics were varied but always contiguous along the IBI range. Variance ratios above the median value were used to identify sensitivity ranges (ratios underlined in Table 9); ratios above the median have a greater effect on the IBI values in those class intervals than those below the median. Abundance of native species (NNAT) affected the IBI value most in the four categories Excellent to Poor; "intolerant" species richness and percent piscivore biomass affected Excellent to Fair; and nonindigenous species richness and percent specialist biomass affected Fair to Very Poor. Centrarchid and native cyprinid species richnesses

TABLE 10. IBI values obtained within a span of a few days at transect locations in the Bay of Quinte and Hamilton Harbour in 1989 and from Matchedash Bay in 1990 (asterisk marks the pair with the geatest absolute difference).

| | | IBI values | | |
|---|---|---|---|---|
| Data set | Transect | Sample 1 | Sample 2 | Sample 3 |
| BQ89 | 11 | 61.5 | 45.8 | — |
| | 15 | 57.9 | 59.1 | — |
| HH88 | 1 | 5.6 | 13.5 | — |
| | 17 | 29.4 | 16.5 | 21.7 |
| | 21 | 19.0 | 35.8 | — |
| | 23* | 12.6 | 53.7 | — |
| | 25 | 22.0 | 21.4 | 27.4 |
| MB90 | 7 | 69.6 | 66.0 | — |
| | 9 | 63.9 | 55.6 | — |

affected Good and Fair, while percent generalist biomass, biomass of native species, and percent of nonindigenous species by numbers and biomass affected Fair and Poor. The number of metrics affecting a single category ranged from two for Very Poor to 12 for Fair.

### Variability of IBI Values

The statistical assessment of IBI values began with testing of the 654 values for goodness of fit with a normal distribution; the K–S $D$ statistic equalled 0.078 (significant at $P = 0.001$), rejecting the null hypothesis. We transformed the IBI values by taking the square root and repeated the K–S test; the $D$ statistic equalled 0.039 and was not significant ($P = 0.268$). The square-root transformation of IBI was used in all subsequent statistical testing.

Across the seven data sets, 117 transects had been sampled three or more times; in Hamilton Harbour in 1989, sample sizes were typically 7–11; elsewhere (Bay of Quinte, Penetang Harbour, and Matchedash Bay in 1990), sample sizes were usually three and occasionally four. Standard errors (SEs) of $\sqrt{\text{IBI}}$ ranged from 0.09 to 2.70 with a mean of 0.60 (Fig. 3). Of the 177 SE values, 59% were less than 0.5 and 87% were less than 1.0. Of the 15 SE values greater than 1.0, six sites had a zero value in their data set (three from BQ90 and three from PH90) and four sites had data sets with a pair of similar values and an outlier. Hamilton SEs were generally higher in 1990 than in 1988, reflecting the different sample sizes (Fig. 3; Table 1). Penetang Harbour and Matchedash Bay SEs were generally lower than those elsewhere, at high mean $\sqrt{\text{IBI}}$s. Bay of Quinte SEs were much more variable accounting for most high values.

As shown by Karr et al. (1986, 1987) in previous IBI derivations, SEs were negatively correlated with $\sqrt{\text{IBI}}$ means ($r = -0.420$ significant at $P = 0.01$ on 116 df); variability of IBI values is greatest where ecosystem conditions are least suitable. SEs were also negatively correlated with number of samples ($r = -0.248$ significant at $P = 0.01$ on 116 df). A multiple regression of SEs with means and sample sizes was significant ($F_{\text{regr}} = 48.97$ significant at $P = 0.001$ on 2,114 df) with both negative slopes being significantly different from 0 ($t$-values = 9.21 and 7.78, respectively, significant at $P = 0.001$ on 114 df). The regression equation was

$$\text{SE} = 2.904 - 0.266 \cdot \text{Mean} \sqrt{\text{IBI}} - 0.130 \times \text{Number of samples.}$$

This result was used to determine required sample sizes for site-to-site comparisons. To estimate the sample size needed to obtain an index of precision (D) = 0.20 (Elliott 1977), we substituted the regression equation into the equation D = SE/mean. The sample size is a simple function of the mean:

$$N = 22.34 - 3.59 \cdot \text{Mean} \sqrt{\text{IBI}} \text{ for } D = 0.2.$$

For a mean of 5 (IBI = 25), the sample required equals five and is lower for higher means. Alternatively, we might expect the IBI to have a Poisson distribution, since the underlying metrics are linear functions of catch data which are usually expected to be Poisson (Elliott 1977). Assuming the IBI values were Poisson, we used another form of $D$ to estimate sample size (Elliott 1977): $D = 1/\sqrt{(N \cdot \text{Mean})}$. This gave similar results for $D = 0.20$:

| | | | |
|---|---|---|---|
| Mean | 1 | 5 | 10 |
| Sample | 25 | 5 | 3 |

Since 116 of 117 $\sqrt{\text{IBI}}$ means exceeded four, a sample size of three to five would suffice in most instances for transect comparisons.

Nine transects were sampled two or three times within a span of a few days (Table 10), mostly in Hamilton Harbour in 1988. These sets of samples provided the closest possible approximation of duplicates. Duplicates are difficult to obtain given the disturbance delivered to the fish assemblage at a site by the passage of an electrofishing boat. Absolute differences of 13 pairs had a mean of 0.97 with a SD of 0.98. With the highest difference (3.78 at transect 23 HH88) excluded, as it appeared to be an outlier, the absolute differences averaged 0.73 with a SD of 0.51. The SE for the mean absolute difference was 0.15, indicating a level of variability lower than that obtained in sets of samples taken over a period of several months. This result suggests that a large portion of the seasonal variability at individual transects is attributable to seasonal fish assemblage changes rather than sampling variability.

At transects 17 and 25 in Hamilton Harbour, three samples were obtained within a short span in 1988 and had mean $\sqrt{\text{IBI}}$s of 4.72 and 4.69, respectively, and SEs of 0.39 and 0.19. These values represent index of precision values of 8.3 and 3.9%, respectively, less than 10%. Again, this suggests that less of the seasonal transect variability is attributable to sampling error.

Paired-transect comparisons between years in Hamilton Harbour and the Bay of Quinte produced contradictory results (Fig. 4). These tests were confounded by two main factors. First, in the Bay of Quinte, single values from one year were compared with the mean of three or four values from another year, placing limits on the amount of possible agreement. Second, the null hypothesis of agreement incorporated the assumption that the sites were the same in the two years. Scheffer et al. (1992) found considerable variability in the presence–absence of two *Potamogeton* species by site over a 20-yr span in a series of six Dutch lakes. Overall, the Pearson correlation between all pairs was significant ($r = 0.71$ significant at $P < 0.01$ on 69 df). In Hamilton Harbour alone, the correlation for 40 transects was significant ($r = 0.51$ significant at $P < 0.01$ on 39 df). If we restricted the transects to those with a sample size >1 in both years, the correlation rose to 0.59 (significant at $P < 0.01$ on 19 df). In the Bay of Quinte, the correlation for

20 transects was 0.14 (not significant at $P = 0.05$). If we excluded the transects where SEs exceeded 1 in 1990, the correlation rose to 0.20, also not significant at $P = 0.05$ on 14 df. The Bay of Quinte comparison was limited by the lack of repeat sampling in 1989. Macrophyte surveys were conducted along 12 of the transects in both years and the Pearson correlation between arcsine ($\sqrt{}$ cover) values was 0.74 (significant at $P = 0.01$ on 11 df). This level of habitat agreement between different years sets an upper limit on IBI agreement given the dependence of IBI on cover shown in the next section.

## Comparisons with Ecosystem and Habitat Conditions

To begin, we performed tests to compare ecosystems without regard to transect-to-transect habitat differences before relating IBI values on particular transects to specific habitat conditions. We performed a one-way analysis of variance on $\sqrt{}$ IBI values, taking the seven data sets as treatments. Ecosystem means were significantly different ($F = 70.1$ on 6,647 df, $P$ <0.05). A Student–Newman–Keuls (SNK) range test (error mean square = 2.172, $P = 0.05$) produced the following results:

| Site | HH90 | HH88 | BQ90 | PH90 | HB90 | MB90 | BQ89 |
|---|---|---|---|---|---|---|---|
| Mean | 5.10 | 5.23 | 7.27 | 7.43 | 7.63 | 8.01 | 8.19 |
| Nonsignificant ranges | a | a | b | bc | bc | bc | c |

Mean values for Hamilton Harbour in 1988 and 1990 were not significantly different (a) and both were different from all other means. The means for Bay of Quinte in 1989 and 1990 were different but neither mean was different from those of the three Severn Sound sites (b and c). In the Bay of Quinte, if we compare the means for August in 1989 and 1990, 8.19 versus 8.14, we find that they are not different.

The values of the offshore metric, $P_{\mathrm{OFF}}$, were generally low in Severn Sound in 1990 with means of 3.8, 0.8, and 6.2% for Penetang Harbour, Hog Bay, and Matchedash Bay, respectively. In the Bay of Quinte, values were higher with means of 27.9 and 17.0% in 1989 and 1990, respectively. Mean values were highest in Hamilton Harbour with means of 46.4 and 42.2% in 1988 and 1990, respectively. One-way analysis of variance on the $\sqrt{}$ IBI* values showed significant differences among means ($F = 107.77$ on 6,647 df, $P = 0.01$). Another SNK test shows that the difference between 1989 and 1990 means in the Bay of Quinte was due to differences in offshore incursions, particularly of walleye, which inflated index values:

| Site | HH90 | HH88 | BQ90 | BQ89 | PH90 | HB90 | MB90 |
|---|---|---|---|---|---|---|---|
| Mean | 3.48 | 3.51 | 6.54 | 6.90 | 7.27 | 7.60 | 7.74 |
| Nonsignificant ranges | a | a | b | bc | bc | bc | c |

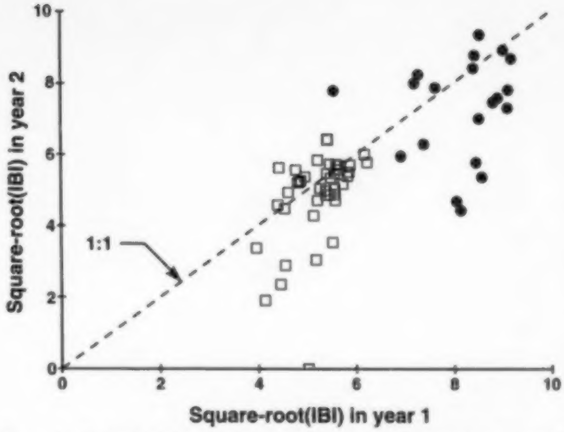

FIG. 4. Relationship between pairs of mean and/or single $\sqrt{}$ IBI values obtained at individual transect in different years: 1988 versus 1990 in Hamilton Harbour (□) and 1989 versus 1990 in the Bay of Quinte (●).

The differences between mean $\sqrt{}$ IBI and $\sqrt{}$ IBI* values show the importance of incursions of offshore species into the littoral zone. This result might suggest a need to incorporate $P_{\mathrm{OFF}}$ into the IBI. As Karr (1981, 1991) explained, the IBI is expected to reflect a range of factors influencing fish assemblages. Overall the IBI should be insensitive to nearshore or offshore habitat preferences of each species, as the total integrity of the fish assemblage is being measured. However, to show the influence of particular factors, i.e., littoral vegetation cover, adjustments, such as $P_{\mathrm{OFF}}$, are needed to partition the IBI. Adding an "offshore" metric to the index would be ineffective. Individual species with offshore preferences or characteristics are already marked as positive or negative indicators in other metrics, i.e., piscivores versus nonindigenous species, and pooling them in an offshore metric would be similar to using total numbers or species richness as a metric. The differences among the AOCs strongly imply that increased incursion by "offshore" fishes is a consequence and symptom of the degradation of littoral fish assemblages and habitats.

Stem density of submerged vegetation was estimated at 72 transects in 1990 among the five sites visited (Table 2). Percent vegetation cover was estimated on 68 of the 72 transects. Across the five sites, Pearson correlations between $\sqrt{}$ IBI and, arcsine($\sqrt{}$ cover), and ln(stems + 1) were significant ($P < 0.01$) (Table 11). Correlations with $\sqrt{}$ IBI* were also significant and greater than those with $\sqrt{}$ IBI. The best result ($r = 0.70$) was between $\sqrt{}$ IBI* and arcsine($\sqrt{}$ cover) (Fig. 5). Regression modelling showed that IBI values were significantly ($R^2 = 0.64$, $F_{\mathrm{regr}} = 87.74$, $P < 0.001$) lower in Hamilton Harbour than at other sites for given cover levels:

$$\sqrt{\mathrm{IBI}^*} = 5.87 - 2.65^* \text{ (1 for HH, 0 for other sites)} + 1.50 \cdot \mathrm{arcsine}(\sqrt{\mathrm{cover}}).$$

Analysis of covariance of $\sqrt{}$ IBI* values from the five sites surveyed in 1990 indicated that (i) percent cover was a significant covariate ($F = 22.10$ significant at $P = 0.000$ on 1,62 df), (ii) the cover slopes were not significantly different ($F = 0.31$ not significant at $P = 0.87$ on 4,58 df), and (iii) there were differences among adjusted data set means ($F = 12.06$ significant at $P = 0.00$ on 1,62 df). Posteriori testing, Bonferroni-adjusted, of least differences between pairs of means showed that Hamilton Harbour had a lower adjusted mean and that there were no differences ($P = 0.01$) among the other four sites. The cover-adjusted mean in Hamilton Harbour was 4.32 (SD = 1.27), while means for the other four sites ranged from 6.40 to 7.10 (0.65–1.73). The overall analysis of covariance model accounted for 84.6 of total variance. Visual inspection of the IBI to cover rela-

TABLE 11. Relationships between IBI and IBI* and submerged macrophytes (percent cover, stem density, biomass, and vegetation class) among the seven site–year data sets. ** Significant at $P = 0.05$; letters signify significant groupings of treatments using $\sqrt{\text{IBI*}}$.

| Data set and habitat variable | $N$ | $\sqrt{\text{IBI}}$ | $\sqrt{\text{IBI*}}$ | |
|---|---|---|---|---|
| 5 sites in 1990 | | Correlation coefficient | | |
| Arcsine ($\sqrt{\text{cover}}$) | 68 | 0.64 | 0.70 | |
| ln(stems + 1) | 72 | 0.46 | 0.61 | |
| Bay of Quinte, 1989 | | | | |
| Arcsine ($\sqrt{\text{cover}}$) | 30 | 0.04 | 0.53 | |
| ln(biomass + 1) | 30 | 0.02 | 0.50 | |
| Hamilton Harbour, 1988 | | Class means | | NS at $P = 0.05$ |
| Absent | 14 | 4.92 | 3.05 | a |
| Sparse | 8 | 5.39 | 2.24 | b |
| Moderate | 10 | 5.16 | 4.13 | c |
| Heavy | 8 | 5.70 | 4.68 | c |
| *F* value, ANOVA, 3,36 df | | 5.08** | 19.40** | |

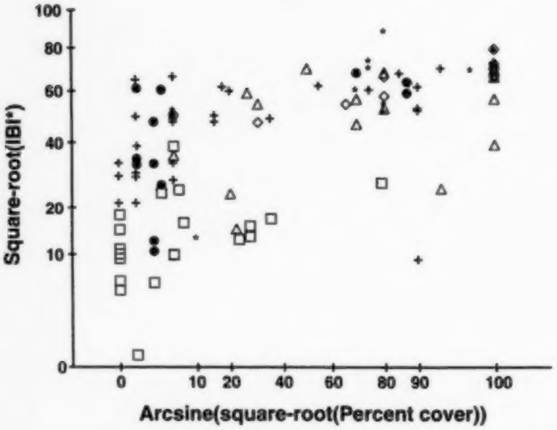

FIG. 5. Relationship between $\sqrt{\text{IBI*}}$ and arcsine-square-root percent submerged plant cover in survey areas: Hamilton Harbour 1990 (□), Bay of Quinte 1990 (●), Penetang Harbour 1990 (△), Hog Bay 1990 (*), Matchedash Bay 1990 (◇), and Bay of Quinte 1989 (+).

tionship (Fig. 5) suggested a slight downturn of IBI values with cover values close to zero. We repeated the analysis of covariance with arcsine($\sqrt{\text{cover}}$) and arcsine$(\sqrt{\text{cover}})^2$ as covariates and the squared term did not enter the analysis significantly ($P > 0.05$). $\sqrt{\text{IBI*}}$ increased linearly with arcsine($\sqrt{\text{cover}}$) on two parallel lines, one for Hamilton Harbour and one for all other sites.

In the 1989 Bay of Quinte results, the relationship between cover and IBI* was similar to that seen for four sites, other than Hamilton Harbour, in the 1990 results (Fig. 5). Analysis of covariance comparing 1989 and 1990 values for the Bay of Quinte indicated that slopes were homogeneous ($F = 0.72$ not significant at $P = 0.40$ on 1,40 df), cover was a significant covariate ($F = 16.13$ significant at $P = 0.00$ on 1,41 df), and adjusted means were not significantly different ($F = 0.30$ not significant at $P = 0.59$ on 1,41 df). In the Bay of Quinte, the relationship between IBI and cover was the same in 1989 and 1990.

We used one-way analysis of variance by submerged vegetation density class to assess variation in IBI and IBI* for transects surveyed in Hamilton Harbour in 1988. The *F* values were significant ($P < 0.05$) for both IBI and IBI* but the latter was much greater (Table 11). A SNK test showed that the mean IBI * values were significantly ($P < 0.05$) different between absent, sparse, and moderate + heavy categories but not between moderate and heavy. Assuming the vegetation classes in 1988 spanned the same ranges of percent cover observed in 1990 in Hamilton Harbour, the pattern and levels of IBI* values were similar in both years (Table 11; Fig. 5).

We used the 1990 data from five sites to examine the expectation (Karr 1981, 1991) that individual metrics might be more highly correlated with habitat than the overall index. For both ln(stem density + 1) and arcsine($\sqrt{\text{cover}}$), only the centrarchid species richness metric, SCEN, had higher correlations, 0.64 and 0.76, respectively, than those with $\sqrt{\text{IBI*}}$. Correlations with the metric for number of native species, 0.47 and 0.65, were greater than those with $\sqrt{\text{IBI}}$. In addition, correlations of stem density with the metrics for numbers and biomass of nonindigenous species, 0.58 and 0.49, respectively, were greater than that with $\sqrt{\text{IBI}}$.

Finally, the individual IBI values from the seven data sets were tabulated by the named categories and percent frequencies calculated. The results show the overall consistency between years in both Hamilton Harbour and Bay of Quinte and emphasize the differences among the five sites (Table 12). Hamilton Harbour is the site least suited to the needs of a healthy fish assemblage; in both years, 56% of samples are rated poor and 22% very poor. The Bay of Quinte data sets have the highest proportions of samples rated excellent, followed by Hog Bay. The differences between the Bay of Quinte and Severn Sound are primarily due to the greater abundance of piscivores although, in Quinte, walleye, an offshore but desirable piscivorous species, accounts for much of that component. In Severn Sound, the

TABLE 12. Percent frequency distributions of IBI values assigned to named categories for data sets collected in the Bay of Quinte, Hamilton Harbour, and Severn Sound.

| | IBI category | | | | | |
|---|---|---|---|---|---|---|
| Location and year | No Fish | Very Poor | Poor | Fair | Good | Excellent |
| Bay of Quinte, 1989 | 0.0 | 0.0 | 2.9 | 23.5 | 52.9 | 20.6 |
| Bay of Quinte, 1990 | 5.1 | 5.1 | 3.4 | 35.6 | 32.2 | 18.6 |
| Hamilton Harbour, 1988 | 1.2 | 22.1 | 56.4 | 20.2 | 0.0 | 0.0 |
| Hamilton Harbour, 1990 | 2.4 | 22.3 | 56.5 | 17.6 | 1.2 | 0.0 |
| Penetang Harbour, 1990 | 3.6 | 0.0 | 3.6 | 44.0 | 45.2 | 3.6 |
| Hog Bay, 1990 | 3.6 | 0.0 | 3.6 | 35.7 | 42.9 | 14.3 |
| Matchedash Bay, 1990 | 0.0 | 0.0 | 5.3 | 23.7 | 68.4 | 2.6 |

distributions of ratings suggest a gradation improving from Penetang Harbour through Hog Bay to Matchedash Bay, consistent with the overall ranked assessments of ecosystem status in those areas. The distributions for the Bay of Quinte were somewhat anomalous because of the influence of offshore species. The distributions of IBI* values have patterns intermediate between those of Hamilton Harbour and Severn Sound, in line with the ordering of areas based on an overall assessment of ecosystem degradation (Randall et al. 1993).

## Discussion

Discussion of these results is divided into three sections: (i) evaluation of the structure and performance of the IBI itself, (ii) assessment of the results with regard to the impairment of the AOCs and the understanding of the processes shaping their fish assemblages, and (iii) speculation about the further development and application of an IBI in Great Lakes littoral habitats.

### Appraisal of IBI Structure and Performance

Karr et al. (1986) showed how the stream IBI met six validation criteria for biomonitoring indices; the index must be (i) biological, (ii) interpretable across many trophic levels, (iii) sensitive to ecosystem status, (iv) responsive over its range to intended uses, (v) reproducible and precise within the constraints of the data collection procedure, and (vi) have low variability. This littoral IBI satisfied these criteria. The index was based entirely on metrics derived from attributes of standardized samples of Great Lakes littoral fish assemblages. Three of 12 metrics are based on the trophic composition by biomass, representing piscivores, generalists, and specialists. The analyses within and between ecosystems showed that the index responds to both the general ecosystem quality status (Hamilton Harbour was different from the other sites) and to a specific habitat variable, macrophyte cover. Analyses of the variability within transect locations showed that index values are reproducible, both seasonally and between years, and that an acceptable level of precision can be attained with modest sample sizes (three to five per transect).

While there is evidence that this lentic IBI meets the criteria erected for lotic IBIs, we must point out a number of differences and difficulties as well as review the advantages and disadvantages outlined by Fausch et al. (1990). In the Great Lakes context, richness and abundance metrics did not need to be standardized for ecosystem size as has been done for lotic metrics using drainage area or stream order (Karr et al. 1986; Miller et al. 1988). Most of the species present in and throughout the adjacent Great Lake have been captured, with one of many fishing gears, in each of the AOCs studied.

In lotic IBIs, fish abundances are used for most, or all, of the nonrichness metrics. Here, 5 of 12 metrics use a biomass base, as the size variation among species is much greater in lakes than in streams. Schlosser (1990) showed a gradient of fish sizes and longevity in lotic ecosystems, shifting from dominance by small short-lived species in headwater streams to larger long-lived ones downstream. This pattern is coupled with trends from lower species richnesses and fish densities toward higher values and toward a wider range of sizes and abundances (cf. Table 1). This overall pattern extends into lacustrine environments where fish species sizes and longevities are similar to those in the lower, wider, deeper reaches of rivers.

The subjectivity of metric selection and of some metric definitions is a major unresolved issue for all IBIs. The three broad areas, species richness, trophic composition, and abundance and condition, are an accepted framework for metric selection and balance (Karr 1981, 1991; Fausch et al. 1990). The selection of species richness metrics is largely determined by the taxonomic diversity of the assemblages. Here the richness metrics were mostly the same as those in Karr's selection. As in other studies (cf. Karr 1991), native species richness (SNAT) was the first choice and, because of the extent of introductions and invasions into the Great Lakes, nonindigenous species richness (SNIN), as a negative metric, a logical second choice. Centrarchid and native cyprinid species richnesses have been used as metrics in many IBI studies. Both groups are well represented in the Great Lakes and their presence in greater richness and abundance is a sign of maturer, stabler ecosystem conditions (Odum 1969) and of greater resource and habitat heterogeneity or biodiversity (Eadie and Keast 1984).

As with most other IBIs, the species richness of turbidity-intolerant forms (SINT) was included here because increased turbidity is a characteristic of ecosystem degradation in Great Lakes' AOCs. The increased turbidity results from increased soil erosion in the catchment, urban runoff, and disturbances of bottom sediments and is usually associated with losses of emergent and submergent vegetation in littoral areas. The intolerant metric is weak because few species were assigned to the group, given the limited anecdotal information. Recent work by Huber and Rylander (1992) on the relationship between turbidity preference and brain morphology in 51 cyprinid species indicates that a more objective, morphometric basis of assigning species to the intolerant metric might be developed in the future.

The trophic composition metrics were similar to those used in other IBI indices although percentage composition by biomass was used instead of numbers because of the large size range of littoral fish species, e.g., carp versus emerald shiner. The impact of species on energy flow in ecosystems is related more to biomass than to abundance. The three categories used, piscivore, generalist, and specialist, differed from those generally used in stream IBIs. Trophic guild memberships have often been presented as objective fact in stream IBIs. In reviewing summaries of diet preference (Carlander 1969; Scott and Crossman 1973; Portt et al. 1988), we found that the information was often anecdotal and contradictory. The wide size ranges encountered within and among littoral fish species assemblages added to the uncertainly as species often change their dietary preferences as they grow. The omnivore category, requiring a diet of at least 25% plant material, was inappropriate for lacustrine fishes where plants are rarely a major diet item. Having assigned some species to the piscivore group with little difficulty, we distinguished between generalists and specialists. The object was to separate those species consuming a widely varying diet drawn from several trophic levels from those species drawing their food primarily from one trophic level. Rachlin et al. (1989) suggested a numerical method of analyzing fish diet and food resource data to arrive at defensible feeding strategy assignments (specialist versus generalist, selectionist versus opportunist). The necessary diet studies are beyond the resources of most surveys, and besides, feeding patterns can change from time to time and from place to place, making reliable trophic assignments difficult. As with intolerant species, a more objective assignment of species might be based on a consideration of overall lifestyle using measured morphometric characteristics to determine the assignments. Portt et al. (1988) showed that morphometric indices could be used to group a selection of Ontario freshwater fishes. The groupings were related to groupings based on feeding and dietary characteristics, life history measures, and reproductive guilds. Such an ecomorphometric approach may in future provide an alternative strategy for forming multidimensional species guilds. Meanwhile, trophic assignments will remain subjective.

In the abundance and condition metrics group, only the abundance metric overlapped with those used in most lotic IBI studies. A biomass metric was added to complement abundance because of the size variation among species and because biomass is an indicator of energy flow in ecosystems (Odum 1969). Metrics for the native portions of abundance and biomass were used to avoid misleading inferences of "better" ecosystem condition when total biomass or abundance was high. Eutrophication can lead to increased biomass and productivity, and nonindigenous species like carp and alewife can make up a substantial proportion of biomass or abundance in Great Lakes littoral areas. Neither condition is representative of a natural, healthy ecosystem. To account for the largely negative impact of nonindigenous species on native Great Lakes fish assemblages (Emery 1985), we added two negative, proportional metrics indicating the contribution of nonindigenous species to total abundance and biomass. Metrics for hybrids and disease were not included here, as the necessary data were not collected. However, other studies have shown that the incidence of tumours and anomalies is elevated in Hamilton Harbour and low in the Bay of Quinte and Severn Sound (COA 1988, 1989; BOQ 1989).

Redundancy initially may be a concern in the formulation of indices, although Fausch et al. (1990) viewed metric redundancy as a strength of the IBI. Incorporating more components in an index increases the effort required to obtain the information. Correlations among the metrics and with the IBI and the results of the PCA pointed to substantial redundancy in the index. However, the analysis of the range sensitivity of the IBI to various metrics showed that the degree and operational range of contribution of individual metrics varied considerably (Table 9). Further, the redundancy can be considered an essential feature, as the response of individual metrics to a variety of stresses will be uncertain.

The changes in the scaling and formulation of the IBI were made to produce an index which was a continuous variable rather than the integer form developed by Karr. A continuous variable is more amenable to simpler forms of statistical analysis. Setting the maximum to 100 was consistent with popular notions of quality and condition.

The inshore–offshore modifier, $P_{\mathrm{OFF}}$, increased our ability to discern the influence of local habitat conditions on littoral fish assemblages. The increased presence of offshore species may be a consequence or an indicator of degraded littoral fish assemblages. Certainly, the increased presence of alewife (an offshore species) is considered a negative factor, but their effect is already expressed in three nonindigenous metrics. Piscivores, like walleye, are not close-in littoral species but rather intermediate between truly littoral species like northern pike and bass and truly offshore species like lake trout (Kitchell et al. 1977). The presence of piscivores is a sign of a healthy ecosystem but a littoral zone with low levels of littoral piscivores are a result of decreased vegetation and other shoreline impairments cannot be considered healthy.

Metric selection for a lotic IBI is based on a set of assumptions about the response of fish assemblages to ecosystem degradation (Fausch et al. 1990) (Table 2). For richness, numbers of species in specific taxa (e.g., centrarchids or cyprinids) or guilds (e.g., insectivore or turbidity intolerant) or native usually decline with degradation. For trophic composition, proportions of specialist, insectivores, top carnivores, etc., decline, while proportions of omnivores or generalists increase. The proportions of individuals in silt-sensitive reproductive guilds decrease, while those with external evidence of disease, parasitism, or anomalies increase. Native abundance or biomass may decline, while total abundance or biomass may increase as the proportions belonging to introduced species increase. While the details of the metric formulations may differ between lotic and lentic ecosystems, the assumptions about the effects of degradation are applicable to littoral fish assemblages in the Great Lakes. Fish assemblages exist along a continuum of habitats, including streams, rivers, and littoral and profundal zones in lakes, and many of the species are encountered in a range of habitats (Steedman and Regier 1987). Degradative processes such as eutrophication, contamination, habitat loss, overfishing, and invasions of exotic species act differentially by species and, then, are expressed in assemblage attributes (Evans et al. 1987).

### Ecosystem Status within and between AOCs

Demonstrating the agreement between IBIs and measures of other ecosystem features and properties is a necessary step toward building confidence. With lotic IBIs, Karr (1991) cited several instances involving water quality and habitat where agreement with IBI patterns has been good. Leonard and Orth (1986) obtained a significant inverse relationship between IBI and an index of cultural pollution for a connected series of streams in West Virginia. Steedman (1988) showed that IBI values at sites on southern Ontario streams were predictable from an assessment of upstream watershed land use patterns. Recently, Oberdorff and Hughes (1992) applied the IBI concept to lotic ecosystems on the Seine Basin, France, and showed that the IBI values were significantly related to both the upstream catchment area and an independent water quality index. Generally, lotic IBI values should express an upstream catchment-wide integration of ecosystem stresses and disturbances. Similarly, whole lakes integrate their catchment conditions, but within lakes, particular locations, for instance in the littoral zone, will integrate the influence of local in situ habitat conditions, adjacent land use, and the free flow of biotic and abiotic components within the lake. The significant correlations between littoral IBIs and macrophyte cover suggest that the spatial integration is strongly influenced by local conditions. Of course, local conditions may, in turn, have arisen as the result of distant watershed and adjacent land use modifications.

Four classes of factors determine the condition of Great Lakes littoral fish assemblages and are represented in our IBI as (1) nonindigenous, or exotic, species abundance, (2) water quality, (3) physical habitat, and (4) piscivore abundance. Each factor may both influence and be influenced, directly or indirectly, by the others; the presence of piscivores can prevent the expansion of undesirable nonindigenous species, hold down planktivore abundance, and thereby allow zooplankton to keep algal biomass at low levels. In turn, light penetration increases, allowing macrophyte populations, a key element of physical habitat, to grow over greater areas with increased abundance. This, in turn, increases the supply of habitat for littoral piscivores. Conversely, any one or all of overexploitation of piscivores, degradation of water quality by eutrophication and contamination, deliberate or accidental introduction of exotic species, and destruction of habitats for human development can perturb the interactions and establish new, undesirable ecosystem states. This four-factor system was represented in model form by Scheffer (1989), encapsulating a broad consensus for eutrophic, shallow freshwater ecosystems in Holland. In shallow Dutch lakes with high turbidity and algal biomass, and low macrophyte abundance, the fish communities are dominated by larger cyprinids and have few piscivores like pike or zander (*Lucioperca* sp.). In clear lakes, macrophytes dominate, cyprinid biomass is low, and pike biomass is high. Scheffer's numerical modelling supported the view that when nutrient inputs are low, the clear state is the only stable situation, and when nutrient inputs are very high, the turbid state is almost invariably the outcome. At intermediate nutrient levels, either state, clear or turbid, is possible and locally stable. We expect that a similar set of possibilities exists for degraded Great Lakes bays.

The results presented here show that our IBI for littoral fish assemblages was influenced by all four factors. Metrics for piscivores and nonindigenous species were programmed into the IBI and comparisons within and between Great Lakes ecosystems showed significant relationships with physical habitat and water quality. The PCA showed a major contrast between the abundance and diversity of nonindigenous species and the abundance of piscivores. The significant relationship between percent cover of macrophytes and the raw and modified IBI values showed the role of physical habitat. The significant differences between survey areas, even after adjustment for cover, demonstrated the importance of the general water quality conditions. We suggest that the Dutch shallow lake model can be applied to the littoral regions and bays of the Great Lakes. The eutrophication of Great Lakes' AOCs raised nutrients to levels allowing for the possibility of both clear and turbid states. The destruction of physical habitat, the deliberate introduction of species like common carp along with human-assisted invasions of alewife and rainbow smelt, and the heavy exploitation of many piscivores created the conditions favourable to the turbid state. Hamilton Harbour and the Bay of Quinte are definitely in that turbid state, although piscivore, albeit offshore, levels are higher in the Bay of Quinte. In the Bay of Quinte, clearer waters are a preferred ecosystem state but that will likely lead to a decrease in walleye's contribution to piscivore biomass, as visual predators like pike and bass are favoured. The bays of Severn Sound are either still in the clear state or, as is likely in the case of Penetang Harbour, perilously close to the transition from clear to turbid.

## Scope for Future Expansions and Applications

The littoral IBI presented here is a logical expansion on those developed for lotic ecosystems. Application to a wider range of Great Lakes littoral habitats is needed to establish the robustness of the index. Other littoral areas on the Great Lakes can be examined, e.g., other AOCs, undeveloped embayments and marshes, and open-lake, exposed shorelines. The present study sites border sedimentary land areas, and thus sites on the granitic Shield need to be tested. Application in inland lakes will require substantial metric revisions, as the number of species found per lakes is typically much lower than in Great Lakes littoral habitats (Minns 1989).

Wider use will probably require that the limits for some metrics be adjusted and, perhaps, a change in the array of metrics. Other fish, fish habitat, and fishery factors might be used to expand the array of IBI metrics. The number of species "associated" with vegetation might be a candidate given the relationships with cover seen here, although it would combine both "good" and "bad" species like pike and carp. Conservation of rare and endangered species was not addressed but needs attention. In other IBIs, metrics for exploitable species and fishable stocks have been used to reflect human interests but such metrics do not assess intrinsic ecosystem features. Percent piscivore biomass (PPIS) already reflects some of these interests indirectly. Adding metrics related to human use of fish resources or species conservation priorities detracts from the primary purpose of an IBI: assessing the intrinsic ecological integrity (beneficial ecosystem uses in the wording of the Great Lakes Water Quality Agreement of 1987) of whole fish assemblages. A preferable approach might be to devise complementary indices for usage and conservation, recognizing discrete, but compatible, ecosystem and human use objectives.

## Conclusions

An IBI has been developed for lower Great Lakes littoral habitats adjacent to sedimentary landforms. There is scope for further refinement of the metrics, development of complementary use and conservation indices, and application to all Great Lakes littoral areas.

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# Bayesian and Related Approaches to Fitting Surplus Production Models

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The Schaefer surplus production model relates equilibrium yield to fishing effort and can be fitted using just information on catch and fishing effort. Sometimes, the fitted model predicts a maximum sustainable yield (height of the parabola) that is clearly unrealistic. In this case, one may wish to use prior information on maximum sustainable yield either to constrain the height of the parabola or to provide a prior distribution for Bayesian estimation. To construct a Bayes estimator, one would generally specify a noninformative prior on the residual error variance and, possibly, on the width of the parabola; the prior distribution for height could be obtained by examining fisheries for similar stocks or species on a per unit area basis. Another possibility is to use an empirical Bayes estimator when data from several fisheries (e.g., individual lakes) are available for several years. The methodology is illustrated on catch and effort data for bigeye tuna (*Thunnus obesus*) and Dungeness crab (*Cancer magister*). The approach can be extended to other fishery models, including nonequilibrium production models. The prior distribution parameters can be allowed to depend on covariates.

Le modèle de production excédentaire de Schaefer met en relation le rendement d'équilibre et l'effort de pêche, et on peut l'ajuster en utilisant uniquement des données sur les prises et l'effort de pêche. Parfois, le modèle ajusté prévoit un rendement maximal soutenu (hauteur de la parabole) nettement irréaliste. Dans ce cas, on peut alors se servir des données a priori sur le rendement maximal soutenu pour limiter la hauteur de la parabole ou pour établir une distribution a priori servant à obtenir une estimation bayesienne. Pour créer un estimateur bayesien, il faut généralement préciser des données a priori non informatives sur la variance de l'erreur résiduelle et, probablement, sur la largeur de la parabole; on peut obtenir la distribution a priori concernant la hauteur en étudiant des types de pêche avec des stocks ou des espèces semblables par unité de surface. On peut également utiliser un estimateur bayesien empirique lorsqu'on dispose de données sur plusieurs pêches (p. ex. lacs individuels) pendant plusieurs années. On illustre la méthode au moyen de données concernant les prises et l'effort de pêche du thon ventru (*Thunnus obesus*) et du Crabe dormeur (*Cancer magister*). L'approche peut être appliquée à d'autres modèles de pêche, y compris des modèles de production non à l'équilibre. Les paramètres de la distribution a priori peuvent être conçus pour dépendre de covariables.

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Despite, or perhaps because of, its simplicity, the parabolic surplus production model of Schaefer (1957) is still widely used for fisheries assessment, particularly in cases where little information exists about the fishery. The requisite data are observations on (equilibrium) catches and the corresponding fishing efforts over a number of years. Equilibrium catch in year *j* is assumed to be given by the dome-shaped relationship

$$(1)\qquad y_j = \beta_1\chi_j - \beta_2\chi_j^2 + \epsilon_j$$

where $y_j$ is the observed catch (at equilibrium) in year *j* corresponding to the fishing effort of $\chi_j$, $\beta_1$ and $\beta_2$ are regression coefficients assumed to be positive, and $\epsilon_j$ is a random error with expectation zero. The height of the parabola is known as the maximum sustainable yield (MSY). Several other production models have been devised which also have minimal data requirements (e.g., Fox 1970; Csirke and Caddy 1983; Schnute 1989). The methods developed in this paper can be modified for these models in a straightforward manner.

It sometimes happens that the predicted height of the parabola is unreasonable. In this case, one may need to use "prior" information from similar situations to improve the estimation (see Walters 1986 for an interesting discussion of the basis for this). Information will rarely be available on the likely values of $\beta_1$ and $\beta_2$ (cf. Hoenig and Hoenig 1986). However, there may be information on the height of the parabola. In many freshwater systems, the MSY can be estimated, albeit crudely, from empirically derived models such as the morphoedaphic index (cf. Ryder 1965; FAO 1980; Hanson and Leggett 1982; and references therein). Attempts have also been made to summarize yields per unit area that can be expected for various marine systems (Marshall 1980; Garcia and LeReste 1981, p.161–163). The yields of similar stocks or species can be expressed on a per area basis and plotted against effort per unit area to obtain a "composite" stock production model. The height of the composite curve provides a rough idea of the MSY per unit area (Munro and Thompson 1973; Marten and Polovina 1982; see also Hoenig et al. 1987 for a recent review).

Information on the width of the parabola for a given stock may be problematical. In some cases, however, comparisons may be made with similar stocks in other lakes or bays.

To utilize prior information on the MSY, it is convenient to reparameterize equation (1) in terms of the height and width of the parabola. Let $h$ be the height and $w$ the half width at $y = 0$; thus, $w$ is the fishing effort producing the MSY, often referred to as $f_{\mathrm{msy}}$. Then, equation (1) can be written as

$$(2) \qquad y_j = \frac{2h}{w} x_j - \frac{h}{w^2} x_j^2 + \epsilon_j .$$

In this paper, we begin by fitting the production model by fixing the height. This leads to consideration of a Bayes estimator. Empirical Bayes estimators are then described for multiple stocks by way of the computational procedures of Dempster et al. (1981) which make use of the expectation–maximization (EM) algorithm. Finally, the methodology is illustrated with the Schaefer model applied to a bigeye tuna (*Thunnus obesus*) stock (Bayes estimates) and to multiple stocks of Dungeness crab (*Cancer magister*) (empirical Bayes estimates).

Bayesian estimation has not been commonly used in fisheries work, there being perhaps two dozen examples in the literature. The underlying philosophy of Bayesian estimation differs fundamentally from that of classical inference and there exists a substantial degree of controversy over the meaning of the differences. The interested reader is referred to Press (1989) for an introduction to Bayesian inference and to Barnett (1982) for a thoughtful comparison of the approaches. The use of Bayesian statistics in a fisheries context is discussed in Walters (1986) and Walters and Ludwig (1994).

## Methodology

### Fishing Effort ($f_{\mathrm{msy}}$) for Given MSY

Suppose that the MSY, $h$, is known. The least-squares estimate of the corresponding fishing effort, $\hat{w}$, is then obtained as the solution of the cubic equation

$$(3) \qquad \hat{w}^3 \sum_{j=1}^{n} y_j x_j - \hat{w}^2 \sum_{j=1}^{n} x_j^2 (y_j + 2h) + 3\hat{w}h \sum_{j=1}^{n} x_j^3 - h \sum_{j=1}^{n} x_j^4 = 0$$

where $n$ is the number of years of data. This equation can be written as

$$a_1 \hat{w}^3 + 3a_2 \hat{w}^2 + 3a_3 \hat{w} + a_4 = 0.$$

Let $H = a_1 a_3 - a_2^2$, $G = a_1^2 a_4 - 3a_1 a_2 a_3 + 2a_2^3$, and $\Delta = G^2 + 4H^3$. If $\Delta > 0$, there exist one real and two complex roots. In this case, Cardan's solution of the cubic is convenient; specifically

$$(4) \qquad \hat{w} = \frac{Z - a_2}{a_1}$$

where

$$Z = Q - \frac{H}{Q} \text{ and } Q = \left( \frac{G - \sqrt{\Delta}}{2} \right)^{1/3} .$$

Let $S^2$ denote the residual sum of squares obtained by estimating $\beta_1$ and $\beta_2$ and, thus, $h$ and $w$, by ordinary, unconstrained least squares, and let $S_h^2$ denote the residual sum of squares obtained by assuming $h$ known. $S_h^2$ cannot, of course, be less than $S^2$. We may readily determine the range of values for $h$, $[h_1, h_2]$ say, for which $S_h^2/S^2 \leq 1 + \delta$, where $\delta$ is some predefined (small) value, for example 0.1. For each chosen value of $h$, there is, by equation (4), a corresponding value of $\hat{w}$. We may thus demarcate a set of sustainable yields and corresponding fishing efforts for which the residual sum of squares is inflated over that obtained by the unconstrained least squares fit by at most $100\delta\%$. We may then ask whether such MSYs, which, in this sense, are consistent with the data, are believable. Conversely, we may judge whether MSYs that we deem feasible are consistent with the data.

Extending this idea, we might place a prior distribution on the MSY. Through equation (4), this will impose a distribution on the corresponding fishing effort. If the relationship between $h$ and $w$ turns out to be essentially linear, then the distribution imposed on $w$ will have essentially the same form as that assumed for $h$ (see the example below).

### Bayesian Estimation

The idea of placing a prior distribution on $h$ leads then to consideration of Bayesian estimation per se.

Assume in equation (2) that the $\epsilon_j$ are independent normally distributed random variables with mean zero and variance $\sigma^2$. The likelihood of the data is then

$$(5) \qquad \Lambda = \left( \frac{1}{\sigma\sqrt{2\pi}} \right)^n \exp\left( -\frac{1}{2\sigma^2} \sum_{j=1}^{n} \left( y_j - \frac{2h}{w} x_j + \frac{h}{w^2} x_j^2 \right)^2 \right).$$

As noted above, we may well have sufficient information to place a prior distribution on $h$ but feel unable to place prior distributions on $w$ and $\sigma^2$. Bayesian estimation, however, requires priors on all unknown parameters. So-called noninformative priors can be placed on $w$ and $\sigma^2$. According to Seber and Wild (1989), the criteria followed by Box and Tiao (1973) lead to the Jeffreys' noninformative prior which here, under the assumption that $w$ and $\sigma^2$ are independent, is given by the product of $|I_1(\sigma)|^{1/2}$ and $|I_2(w)|^{1/2}$ where, with $L = \log(\Lambda)$,

$$I_1(\sigma) = E\left[ -\frac{\partial^2 L}{\partial \sigma^2} \right]$$

$$I_2(w) = E\left[ \frac{\partial^2 L}{\partial w^2} \right]$$

and, in each, the other parameters are treated as known. Thus

$$I_1(\sigma) = \frac{2n}{\sigma^2} \propto \sigma^{-2}$$

$$I_2(w) = \frac{4h^2}{\sigma^2 w^6} \sum_{j=1}^{n} x_j^2 (x_j - w)^2 \propto \frac{\sum x_j^2 (x_j - w)^2}{w^6}.$$

Note that if, as seems reasonable in this and many situations, the fishing gear is not saturated even under the highest catches, *h* may be assumed to be independent of *w*. Then, following Seber and Wild (1989) the posterior probability density of *h*, *w*, and σ, *P*(*h*,*w*,σ|**y**), is proportional to

$$\frac{1}{\sigma^{n+1}}\exp\left(-\sum_{j=1}^{n}(y_j-\hat{y}_j)^2/2\sigma^2\right)$$

$$\times\frac{\sqrt{\sum_{j=1}^{n}x_j^2\,(x_j-w)^2}}{w^3}\,p(h)$$

where $\hat{y}_j=\frac{2h}{w}\chi_j-\frac{h}{w^2}\chi_j^2$ and *p*(*h*) denotes the prior probability density of *h* chosen by the analyst. The above can be integrated over σ to eliminate σ, whence

$$(6)\qquad P(h,w\mid\mathbf{y})$$

$$\propto\frac{1}{w^2}\left[\frac{\sum x_j^2\,(1-x_j/w)^2}{\left[\sum(y_j-2hx_j/w+h^2x_j^2/w^2)\right]^n}\right]^{1/2}p(h)$$

$$=\xi(h,w),\ \text{say}.$$

Next, ξ(h,w) can be integrated numerically over *h* and *w* to determine the constant necessary to obtain a proper posterior probability density. Let $1/\kappa=\int_0^\infty\int_0^\infty\xi(h,w)dh\,dw$; then the posterior joint density for *h* and *w* is

$$(7)\qquad P(h,w\mid\mathbf{y})=\kappa\xi(h,w).$$

The marginal posterior density for *h* can be obtained by integrating equation (7) over *w*; thus

$$P(h\mid\mathbf{y})=\int_0^\infty P(h,w\mid\mathbf{y})dw$$

and the mean of the posterior marginal density is a Bayes estimator of the maximum sustainable yield, i.e.,

$$\hat{h}_B=\int_0^\infty hP(h\mid\mathbf{y})dh.$$

The 100(1 − α)% credibility interval (the Bayesian analog of the confidence interval) is ($h_1$,$h_2$) where

$$\int_0^{h_1}P(h\mid\mathbf{y})dh=\alpha/2$$

$$\int_{h_2}^{\infty}P(h\mid\mathbf{y})dh=\alpha/2.$$

The Bayes estimate for the corresponding fishing effort, *w*, and its credibility interval are obtained from equation (7) in a parallel manner.

Credibility intervals describe the (posterior) degree of belief of the parameter falling in the interval, given the data (Press 1989). Bayesian statisticians find this a natural interpretation for an interval estimate. They point out that, in contrast, the traditional confidence interval has an awkward, frequentist interpretation of being a random interval which, if constructed many times, will include the true value of the parameter a specified percentage of the times.

If it should happen that substantial information exists concerning the fishing effort, *w*, that will result in catches equal to the MSY (i.e., *h*), then one may wish to specify an informative prior distribution for *w*. The Bayesian estimation procedure described above is easily modified to accommodate an informative prior on *w*, say *q*(*w*). The posterior probability density of *h*, *w*, and σ, *P*(*h*,*w*,σ|**y**) is then proportional to

$$\frac{1}{\sigma^{n+1}}\exp\left(-\sum_{j=1}^{n}(y_j-\hat{y}_j)^2/2\sigma^2)\,p(h)q(w)\right)$$

where all symbols are as previously defined. This expression can again be integrated over σ to eliminate σ. The result is proportional to the joint posterior density of *h* and *w* and can be converted to a proper probability density by multiplying by a constant determined as for equation (6). The determination of marginal posterior densities, Bayes estimates, and credibility intervals proceeds as for the case when the prior distribution for *w* was noninformative.

### Empirical Bayes Estimation

Empirical Bayes methods are useful when one has several series of observations on similar situations or similar systems. For example, there may be observations on a stock in each of several similar lakes. If information is strong for a particular stock, the empirical Bayes estimates will be little affected by consideration of information from other stocks. However, if there is little information for a particular stock, consideration of information from other stocks will cause the estimates for that stock to be pulled towards the mean estimates of all stocks. If information is very weak, these "shrunken" estimates may well be better estimates for the particular stock than the estimates based on the information from that stock only.

Let $y_{ij}$ and $x_{ij}$ be the catch and effort, respectively, for fishery (e.g., lake) *i* in year *j* for *i* = 1, 2, ..., *I* and *j* = 1, 2, ..., $n_i$. The analogous model to equation (1) is

$$y_{ij}=\beta_{1i}x_{ij}-\beta_{2i}x_{ij}^2+\epsilon_{ij}$$

where random error terms, $\epsilon_{ij}$, are, again, independent normally distributed random variables with mean zero and variance $\sigma^2$.

Suppose, now, that production parameters for the various stocks arise from a bivariate normal distribution with mean ($\mu_1$,$\mu_2$) and covariance matrix **Σ**. The model can be rewritten

$$(8)\qquad y_{ij}=\mu_1x_{ij}+(\beta_{1i}-\mu_1)x_{ij}-\mu_2x_{ij}^2-(\beta_{2i}-\mu_2)x_{ij}^2+\epsilon_{ij}.$$

Here, $\mu_1$ and $\mu_2$ are regarded as fixed effects, i.e., intrinsic to the species, while $\beta_{1i}-\mu_1$ and $\beta_{2i}-\mu_2$ are regarded as random effects, i.e., varying over the different stocks of the species, with bivariate $N(\mathbf{0},\mathbf{\Sigma})$ distribution.

Dempster et al. (1981) showed that Bayesian estimation of the $\beta_{1i}$ and $\beta_{2i}$ is straightforward when $\sigma^2$ and **Σ** are known. Indeed, estimates of the posterior distribution of $\beta_{1i}$ and $\beta_{2i}$ can be obtained from ordinary least squares algorithms. In the present case, $\sigma^2$ and **Σ** are not known.

The EM algorithm is a useful procedure for finding maximum likelihood estimates when the data are incomplete. In this case, the data are incomplete in the sense that the sufficient statistics for $\sigma^2$ and **Σ** involve not only the observations, $y_{ij}$, but also the unknown regression coefficients, $\beta_{1i}$ and $\beta_{2i}$. The EM algorithm proceeds as follows. Begin with an initial guess of $\sigma^2$ and **Σ**. Given these, compute the expected value of the sufficient statistics for $\sigma^2$ and **Σ** (the expectation step) — this involves computing Bayes estimates of the regression coefficients $\beta_{1i}$ and $\beta_{2i}$ conditional on the [current] values of $\sigma^2$ and **Σ**. Given these estimates of the sufficient statistics, it is an easy matter to compute maximum likelihood

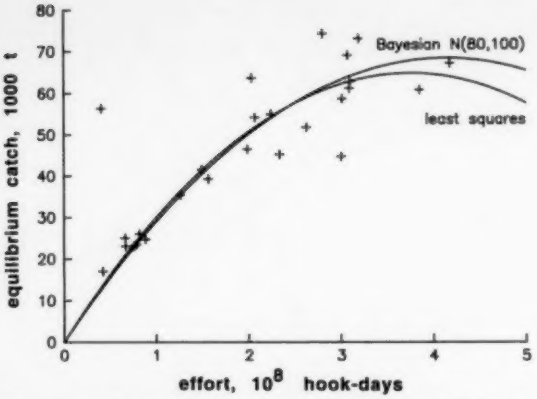

FIG. 1. Scatter plot of catch versus fishing effort for bigeye tuna from the Atlantic Ocean (from Miyabe 1989). Also shown are the Schaefer model (parabola) fitted by ordinary least squares and by the Bayesian method with an $N(80,100)$ prior of height ($h$) and noninformative priors on half-width ($w$) and the residual variance ($\sigma^2$).

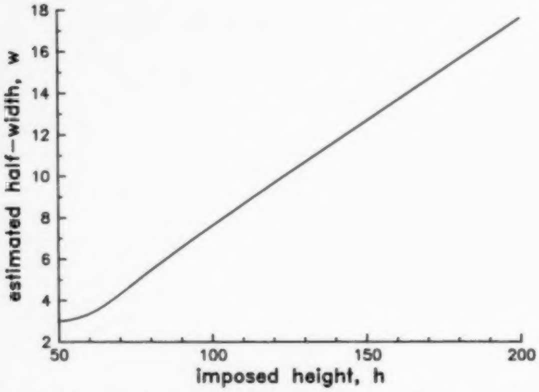

FIG. 2. Relationship between the imposed height ($h$) of the parabola fitted to the bigeye tuna data and the resulting estimated half-width ($w$). Note that the relationship is nearly linear for imposed heights greater than approximately 65 t.

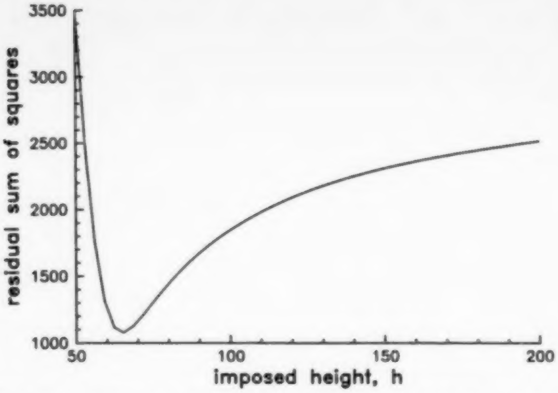

FIG. 3. Relationship between the imposed height ($h$) of the parabola fitted to bigeye tuna data and the resulting residual sum of squares.

estimates of $\sigma^2$ and $\mathbf{\Sigma}$ (the maximization step). The new values of $\sigma^2$ and $\mathbf{\Sigma}$ can then be used to begin the next cycle of iteration, i.e., to update estimates of the expected values of the sufficient statistics so that new maximum likelihood estimates of $\sigma^2$ and $\mathbf{\Sigma}$ can be obtained, and so forth until convergence is achieved. Note that the empirical Bayes estimates of the regression coefficients are obtained automatically as a by-product of the algorithm. Computational details are given in the Appendix.

## Examples

### Optimal Fishing Effort for Given MSY

Catch and effort data for bigeye tuna in the Atlantic Ocean have been tabulated by Miyabe (1989). The observations fall on the ascending (left) limb of the parabola (Fig. 1).

The ordinary least squares (unconstrained) estimates of the parameters are MSY, $h = 64.7 \times 10^3$ t, and corresponding effort, $w = 3.75 \times 10^8$ hook-days, with a residual sum of squares of 1075.9 (Fig. 1). Computation of constrained estimates, i.e., values of the effort for selected values of the MSY (from equation (4)), shows that $w$ is a nearly linear function of the imposed height, $h$, for $h$ greater than approximately $65 \times 10^3$ t (Fig. 2). Accordingly, it is relatively easy to translate one's feelings about the probable value of MSY into feelings about the associated effort. In particular, one could place a probability distribution on $h$ and determine the consequent distribution on $w$.

Suppose now that we wish to consider estimates that inflate the (unconstrained) sum of squares by at most 10%, i.e., find constrained estimates so that the residual sum of squares $\leq 1183.5$. This is achieved with any $h$ in the range $(60.5 \times 10^3, 70.0 \times 10^3)$ (Fig. 3). By equation (4), the corresponding efforts fall in the range $(3.40 \times 10^8, 4.65 \times 10^8)$.

### Bayesian Estimation

The same data are used to illustrate Bayesian estimation per se. Noninformative (Jeffreys') priors are assumed for $w$ and $\sigma^2$ whereas, for illustrative purposes, a normal prior, $N(80,100)$, is assumed for $h$ (note that the prior distribution should be specified without reference to the data). From equation (7) the Bayes estimate of the MSY is then $68.3 \times 10^3$ t with a 95% credibility interval of $(62.2 \times 10^3, 77.4 \times 10^3)$ t. The Bayes estimate of the needed effort is $4.14 \times 10^8$ hook-days with a 95% credibility interval of $(3.42 \times 10^8, 5.21 \times 10^8)$ hook-days. Observe that the Bayes estimate of MSY is moved from the unconstrained least squares estimate towards the mean of the prior distribution. The stronger the degree of prior belief, as reflected by a smaller variance for the prior distribution, the greater the shift towards the prior mean.

### Empirical Bayes Estimates

Computation of empirical Bayes estimates is illustrated with catch and effort data from 12 Dungeness crab fisheries around British Columbia, Canada. A previous analysis of these data has been made by Stocker and Butler (1990). Observations are available for periods ranging from 27 to 43 yr. Following Stocker and Butler, it is logical to consider surplus production models for these fisheries. Equilibrium models should be appropriate for this species for the following reasons: maturity is attained at age 2–3 yr

TABLE 1. Areas inhabited by 12 stocks of Dungeness crab in British Columbia, Canada, and estimates of the production parameters expressed on an areal basis. LS = least squares; EB = empirical Bayes; EBH = empirical Bayes without data from Hecate Strait.

| Stock | Area ($km^2$) | Method | $\hat{\beta}_1$ | $\hat{\beta}_2(\times 10^{-2})$ | $\hat{w}$ ($d/km^2$) | $\hat{h}$ ($t/km^2$) |
|---|---|---|---|---|---|---|
| Boundary Bay | 45 | LS | 0.1463 | 0.0792 | 92.3 | 6.75 |
| | | EB | 0.1463 | 0.0794 | 92.1 | 6.74 |
| | | EBH | 0.1457 | 0.0784 | 92.9 | 6.77 |
| Burrard Inlet | 39 | LS | 0.0856 | 0.0738 | 58.0 | 2.48 |
| | | EB | 0.0857 | 0.0740 | 57.9 | 2.48 |
| | | EBH | 0.0861 | 0.0747 | 57.6 | 2.48 |
| Chatham Sound | 82 | LS | 0.2141 | 0.7665 | 14.0 | 1.49 |
| | | EB | 0.1692 | 0.2749 | 30.8 | 2.60 |
| | | EBH | 0.1640 | 0.2723 | 30.1 | 2.47 |
| English Bay | 52 | LS | 0.0496 | −0.5160 | — | — |
| | | EB | 0.0844 | −0.2551 | — | — |
| | | EBH | 0.0846 | −0.2496 | — | — |
| Fraser River Mouth | 107 | LS | 0.1928 | 0.4065 | 23.7 | 2.29 |
| | | EB | 0.1844 | 0.3590 | 25.7 | 2.27 |
| | | EBH | 0.1807 | 0.3435 | 26.3 | 2.38 |
| Gulf Islands | 62 | LS | 0.0512 | 0.0704 | 36.4 | 0.93 |
| | | EB | 0.0475 | 0.0480 | 49.4 | 1.17 |
| | | EBH | 0.0529 | 0.0717 | 36.9 | 0.98 |
| Hecate Strait | 645 | LS | 1.8958 | 0.2289 | 4.1 | 3.92 |
| | | EB | 1.6256 | 2.0132 | 40.4 | 32.8 |
| Nanaimo | 52 | LS | 0.0479 | 0.0527 | 45.4 | 1.09 |
| | | EB | 0.0464 | 0.0439 | 52.9 | 1.23 |
| | | EBH | 0.0510 | 0.0614 | 41.6 | 1.06 |
| Queen Charlotte Sound | 168 | LS | 0.0851 | 0.3926 | 10.8 | 0.46 |
| | | EB | 0.0612 | 0.0572 | 53.5 | 1.64 |
| | | EBH | 0.0647 | 0.0478 | 67.6 | 2.19 |
| Sidney–Esquimalt | 78 | LS | 0.0618 | 0.0829 | 37.3 | 1.15 |
| | | EB | 0.0609 | 0.0790 | 38.6 | 1.17 |
| | | EBH | 0.0640 | 0.0888 | 36.1 | 1.15 |
| Sooke Harbour | 91 | LS | 0.0506 | 0.0314 | 80.5 | 2.04 |
| | | EB | 0.0508 | 0.0319 | 79.6 | 2.02 |
| | | EBH | 0.0542 | 0.0412 | 65.8 | 1.78 |
| Tofino | 104 | LS | 0.1789 | 0.2234 | 40.0 | 3.58 |
| | | EB | 0.1786 | 0.2224 | 40.2 | 3.59 |
| | | EBH | 0.1750 | 0.2159 | 40.7 | 3.58 |

and males recruit to the fishery at 4–5 yr so that long lags would not be expected in the population's response to changing fishing effort, and the fishery is mainly seasonal so that the population has some time between seasons to respond to such changes.

These crab data have been chosen for the purpose of illustrating the application of empirical Bayes methods to estimate the parabolic form of the Schaefer model (equation (1)). Stocker and Butler (1990) considered several possible error structures and selected a different form of the production model than we used. Consequently, their estimates differ somewhat from those obtained here. The interested reader is referred to their paper for a careful discussion of these data.

Since the production available from a stock is determined, in part, by the size of the stock, it is reasonable to standardize the data by expressing catches and efforts on a per area basis. Accordingly, the catches and efforts have been divided by the fishing area, i.e., the area occupied by, and fished for, each stock (Table 1; T. Butler, 2630 Lynburn Crescent, Nanaimo, BC V9S 3T6, personal communication). This has no effect on the relative form of the fitted models but rescales the data so that the assumption that the parameters, $\beta_{1i} - \mu_1$, $\beta_{2i} - \mu_2$, can be regarded as random variables from a common distribution should be better approximated.

Scatter plots (Fig. 4) reveal much variability in the catches for a given effort, as is typical of production data. Schaefer models (equation (1)) fitted by ordinary least squares appear to give reasonable fits in some cases but unreasonable fits in others (Fig. 4; Table 1). In particular, the fitted parabola for the English Bay data is cup- rather than dome-shaped. This is inconsistent with the assumptions of the Schaefer model. We have elected, however, not to reject the English Bay data in order to see whether the empirical Bayes estimate, which incorporates information from the other stocks, will be feasible. It is noted that Hecate Strait is atypical in that the second highest MSY per square kilometre is attained with the smallest (standardized) effort.

With the model taken as in equation (8), empirical Bayes estimates were obtained by following Appendix equation (A.1) through (A.7) for all 12 stocks. The procedure converged to $\hat{\sigma}^2 = 0.1935$,

$$\hat{\mathbf{\Sigma}} = \begin{bmatrix} 0.1980 & -0.0025 \\ -0.0025 & 0.000034 \end{bmatrix},$$

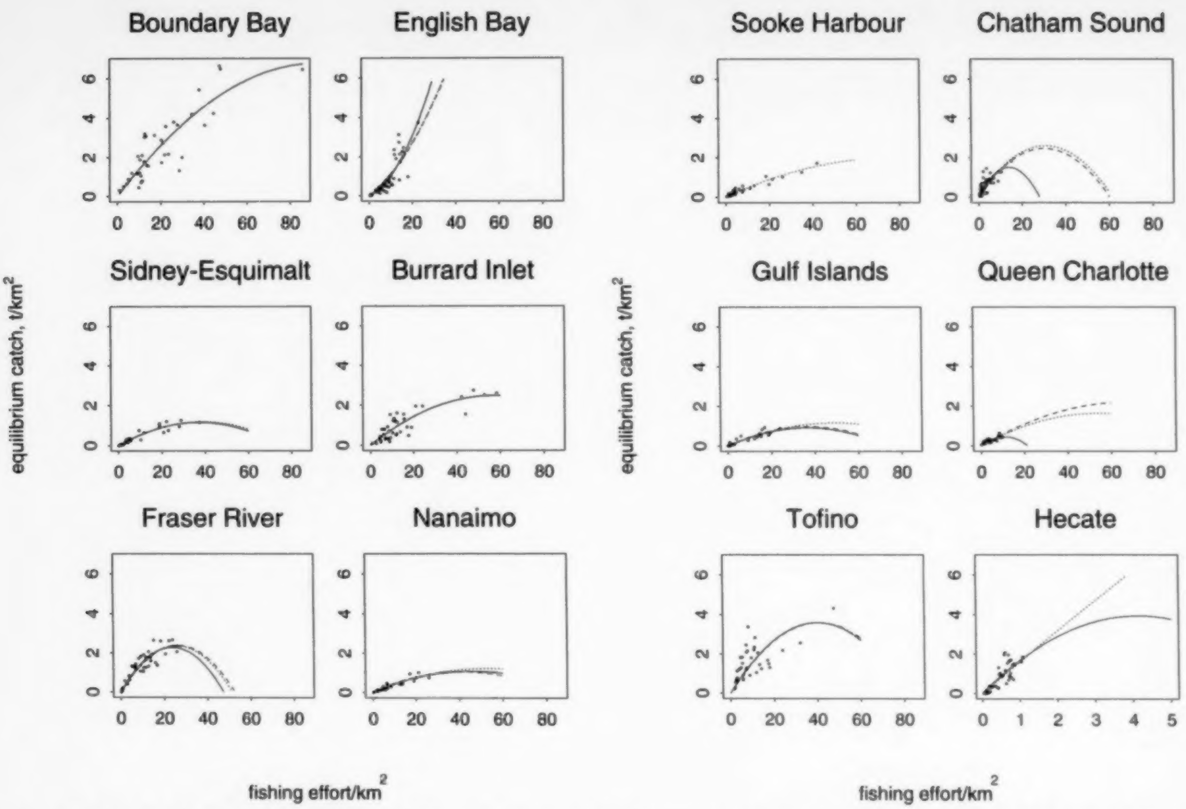

FIG. 4. Scatter plots of catch versus fishing effort for 12 stocks of Dungeness crab from British Columbia, Canada. Also shown are the least squares (solid line) estimates of the Schaefer production model and empirical Bayes estimates with (dotted line) and without (dashed line) the data from Hecate Strait. Note that the scale for the Hecate Strait data differs from the other scales.

TABLE 2. Range of standardized fishing efforts (d/km$^2$).

| Stock | Minimum | Maximum |
|---|---|---|
| Boundary Bay | 1.0 | 86.0 |
| Burrard Inlet | 0.9 | 58.3 |
| Chatham Sound | 0.3 | 15.1 |
| English Bay | 0.4 | 22.7 |
| Fraser River Mouth | 0.1 | 25.9 |
| Gulf Islands | <0.1 | 23.8 |
| Hecate Strait | <0.1 | 1.1 |
| Nanaimo | 0.2 | 26.0 |
| Queen Charlotte Sound | 0.2 | 9.8 |
| Sidney–Esquimalt | 0.1 | 37.4 |
| Sooke Harbour | 0.4 | 41.9 |
| Tofino | 1.6 | 47.6 |

and $\hat{\mu}' = [0.228, 0.00252]$. The estimates of the production parameters are given in Table 1.

For some stocks the differences between the empirical Bayes estimates and the least squares estimates are virtually imperceptible, for example, Boundary Bay, Burrard Inlet, Sooke Harbour, and Tofino. On the other hand, there are stocks, namely Chatham Sound, Queen Charlotte Sound, and Hecate Strait, where the differences are substantial. The similarity, or lack thereof, between the empirical Bayes and least squares estimates is related to the range of fishing efforts (Table 2). It would appear that, for Chatham Sound and Queen Charlotte Sound, the fishing effort employed does not approach that corresponding to the MSY; forcing the Schaefer model on these data leads to seemingly unrealistic least squares estimates whereas the empirical Bayes estimates are much more in accordance with what would be expected in relation to the production of other stocks. The standardized efforts (effort per unit area) for Hecate Strait are so small as to contain virtually no information on the catch–effort relationship. Accordingly, the empirical Bayes procedure takes as estimate of $w$ a value close to the average of the other stocks. Because the catches are relatively high for these small efforts, the empirical Bayes estimate of $h$ is then, seemingly, unrealistically high.

Although moved in the right direction, the catch–effort curve for English Bay remains cup-shaped. The cup-shaped curve for English Bay arises from the English Bay data appearing to fall into two groups. This was noticed by Stocker and Butler (1990) who fitted separate lines to what they judged to be separate phases of the fishery, namely (i) 1963–72 and (ii) the remaining years 1951–62 and 1973–88.

It has been assumed that the $\beta_{1i} - \mu_1$ and $\beta_{2i} - \mu_2$ are bivariate normally distributed. However, one does not need any formal test to conclude that it is inconceivable that the empirical Bayes estimates (Table 3) could arise from a normal distribution. Hecate Strait is clearly an outlier. This

TABLE 3. Ranked empirical bayes estimates of regression parameters.

| Stock | $\hat{\beta}_{1i} - \hat{\mu}_1$ | Stock | $\hat{\beta}_{2i} - \hat{\mu}_2$ ($\times 10^{-2}$) |
|---|---|---|---|
| Nanaimo | −0.182 | English Bay | −0.507 |
| Gulf Islands | −0.181 | Sooke Harbour | −0.220 |
| Sooke Harbour | −0.178 | Nanaimo | −0.208 |
| Sidney–Esquimalt | −0.168 | Gulf Islands | −0.204 |
| Queen Charlotte Sound | −0.167 | Queen Charlotte Sound | −0.195 |
| English Bay | −0.144 | Burrard Inlet | −0.178 |
| Burrard Inlet | −0.143 | Sidney–Esquimalt | −0.173 |
| Boundary Bay | −0.082 | Boundary Bay | −0.173 |
| Chatham Sound | −0.059 | Tofino | −0.030 |
| Tofino | −0.050 | Chatham Sound | 0.023 |
| Fraser River Mouth | −0.044 | Fraser River Mouth | 0.107 |
| Hecate Strait | 1.397 | Hecate Strait | 1.760 |

TABLE 4. Empirical bayes estimates of regression parameters (Hecate Strait omitted).

| Stock | $\hat{\beta}_{1i} - \hat{\mu}_1$ | $\hat{\beta}_{2i} - \hat{\mu}_2$ ($\times 10^{-2}$) |
|---|---|---|
| Boundary Bay | 0.044 | −0.017 |
| Burrard Inlet | −0.016 | −0.020 |
| Chatham Sound | 0.062 | 0.177 |
| English Bay | −0.018 | −0.345 |
| Fraser River Mouth | 0.079 | 0.248 |
| Gulf Islands | −0.049 | −0.023 |
| Nanaimo | −0.051 | −0.034 |
| Queen Charlotte Sound | −0.038 | −0.047 |
| Sidney–Esquimalt | −0.038 | −0.006 |
| Sooke Harbour | −0.048 | −0.054 |
| Tofino | 0.074 | 0.121 |

raises the question of how much influence the Hecate Strait data may have had on the empirical Bayes estimates of the other stocks. Accordingly, empirical Bayes estimates have been computed with the Hecate Strait data excluded (Table 1, with $\hat{\mu}' = [0.102, -0.00311]$). The only notable changes in the estimates occur with Queen Charlotte Sound, Sooke, and, in particular, Gulf Islands and Nanaimo, with the last two moving towards the least squares estimates; in all cases, difference in the fits over the range of the data is virtually imperceptible. For completeness, the estimates of $\beta_{1i} - \mu_1$ and $\beta_{2i} - \mu_2$ are given in Table 4.

## Discussion

A suite of methods for fitting surplus production models has been presented. These methods vary in their data requirements and their ease of computation. The estimator based on fixing the height (equation (4)) is a convenient tool when stock assessment must be done quickly, such as during meetings. The convenience lies in the availability of an analytic solution and in the near linearity of the relationship between the assumed height (or MSY) and the estimated width (or corresponding effort). The other methods require considerably more computation. The Bayes estimates are useful when limited prior information is available to aid in parameter estimation. However, when observations are available on several similar stocks, empirical Bayes estimates become attractive.

A classical alternative, used for example by Polovina (1989), is to fit production models simultaneously to a set of stocks under the assumption that at least one of the parameters is common to all stocks. A special case of this is Munro's composite production model (Munro and Thompson 1973) in which a single parabola is fitted to all of the data after normalizing catches and efforts to a per area basis. This involves fitting fewer parameters than the empirical Bayes approach and may be useful when only a few years of data are available. However, it is not nearly as flexible. One drawback with empirical Bayes estimators is that, as noted by Press (1989), because there is no natural standard error, it is difficult to find credibility intervals or to test hypotheses.

It is well known that, when the necessary assumptions are met, empirical Bayes estimates will be as good as, or better than, the separate least squares estimates for each system. Indeed, Press (1989) observed that "Often, the risk of the empirical Bayes estimator is less than half that of the MLE [maximum likelihood estimator]". Of course, the ordinary least squares estimates are "best" under a simple least squares criterion. In the surplus production example the differences between least squares and empirical Bayes estimates occur primarily outside the range of the data (i.e., in the region where there is little or no direct information). The residual sums of squares from the empirical Bayes estimates are only marginally inflated over those from the least squares estimates. In those cases where least squares estimates of the height and width are poorly defined, they are made more compatible with the estimates for the other stocks; otherwise, they are little, if at all, changed.

The assumptions behind empirical Bayes estimation of the stock production model that might fail are as follows: (i) the $\epsilon_{ij}$ may not be normally distributed, their variance may vary from stock to stock and with effort within a stock, and they may not be mutually independent among years within a stock, (ii) the regression parameters may not be independent random observations from a normal prior distribution, (iii) equilibrium production may be an asymmetric function of effort rather than the parabolic function used, (iv) production may not be at equilibrium in all years, and (v) catchability may be changing (increasing) over time.

The crab example suggests that the results are reasonably robust against violation of (ii) and this assumption can be assessed by looking at the distribution of the regression parameter estimates. Likewise the estimates, although not necessarily their variance, should be reasonably robust against violations of (i). Violation of (iii) would not appear to be a problem if the bulk of the data fall on, and fully cover, the ascending arm of the curve and the second-degree equation is a reasonable approximation for that arm. The English Bay data suggest that violation of (iv) or (v) can, however, be a problem. Nonequilibrium models (not considered here) may be useful for situations where the effort has changed rapidly over time. In rapidly developing fisheries, MSY tends to be overestimated if equilibrium models are used. If the catchability increases over time, then MSY will also tend to be overestimated; this is difficult to handle.

Although we have chosen to illustrate Bayesian approaches to surplus production modelling using the simple equilibrium Schaefer model, the modifications required to handle other production models are in many cases minor. Consider, for example, the nonequilibrium model described by Schnute (1989), namely

$$y_j = (1-\delta)\frac{2h}{w}x_j - (1-\delta)\frac{h}{w^2}x_j^2 + \frac{\delta y_{j-1}x_j}{x_{j-1}} + \epsilon_j$$

where $|\delta| < 1$ and the other symbols are as defined above. This model describes the catch in year $j$ as a weighted mean of the equilibrium production in year $j$ and a residual effect dependent on the catch in the previous year ($j - 1$). It is thus a first-order autoregressive model for which Schnute (1987) shows how to construct the likelihood.

To cast this model in a Bayesian framework, one simply multiplies the likelihood for the data by the prior distributions for each parameter ($h$, $w$, $\delta$, $\sigma$) to obtain a function which is proportional to the joint posterior probability density function. This function is converted to a proper density function by multiplying by a suitable constant (determined by numerical integration). The posterior density is then treated in the same manner as above.

The methods considered in this paper can also be extended to allow the prior distribution of the parameters to depend on covariates. Strenio et al. (1983) described a generalization of the procedures of Dempster et al. (1981) to allow the expected value ($\mu_1$,$\mu_2$) of the production parameters to be linear functions of covariates. Thus, for example, the production parameters might depend on water depth, water temperature, area encompassed by the stock, etc. It seems that lack of data, rather than lack of suitable methodology, is the impediment to future analysis.

Finally, it is noted that the same approach used here for stock production models can be used for other fishery models. In particular, the fit of individual stock–recruitment models is generally quite poor but there may be data available for several stocks. For example, instead of fitting a separate Ricker model to the salmon stock in each stream, one could use an empirical Bayes procedure to fit models to all the stocks simultaneously.

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## Appendix

### Computation of the Empirical Bayes Estimates

Algebraically, the specifics for the situation represented by equation (8) of the main text are as follows. Let equation (8) be expressed in matrix form as

$$\mathbf{y} = \mathbf{X}\boldsymbol{\gamma} + \boldsymbol{\epsilon}$$

where $\mathbf{y}$ is an $n \times 1$ vector of catches ($n = \Sigma n_i$) obtained by concatenating the catches from all $I$ stocks into a single vector and $\mathbf{X} = [\mathbf{B}_x, \mathbf{D}_x]$ where $\mathbf{B}_x = [\mathbf{X}_1', \mathbf{X}_2', \ldots, \mathbf{X}_I']'$, $\mathbf{D}_x = \text{diag}\,(\mathbf{X}_1, \mathbf{X}_2, \ldots, \mathbf{X}_I)$, and

$$\mathbf{X}_i = \begin{bmatrix} x_{i1} & -x_{i1}^2 \\ x_{i2} & -x_{i2}^2 \\ \cdot & \cdot \\ \cdot & \cdot \\ \cdot & \cdot \\ x_{in_i} & -x_{in_i}^2 \end{bmatrix}.$$

Also, $\boldsymbol{\gamma}' = [\boldsymbol{\mu}', \mathbf{v}_\beta']$ where $\boldsymbol{\mu}' = [\mu_1, \mu_2]$, $\mathbf{v}_\beta' = [\mathbf{v}_1', \mathbf{v}_2', \ldots, \mathbf{v}_I']$ and $\mathbf{v}_i' = [\beta_{1i} - \mu_1, \beta_{2i} - \mu_2]$. Note that $\boldsymbol{\gamma}'$ is a $1 \times 2(I + 1)$ row vector. Finally $\boldsymbol{\epsilon}$ is the $n \times 1$ vector of normally distributed residuals assumed to be independent with mean zero and variance $\sigma^2$. In addition, we define the $2I \times 2I$ block diagonal matrix $\mathbf{V}$ as diag $(\boldsymbol{\Sigma}, \boldsymbol{\Sigma}, \ldots, \boldsymbol{\Sigma})$.

To begin the EM algorithm, we need initial guesses for $\mathbf{V}$ (or, equivalently, $\boldsymbol{\Sigma}$) and $\sigma^2$ which we denote by $\mathbf{V}^{(0)}$ (or $\boldsymbol{\Sigma}^{(0)}$) and $\sigma^{2(0)}$, respectively (superscripts in parentheses indicate the number of iterations performed).

The sufficient statistics for $\sigma^2$ and $\mathbf{V}$ are $\boldsymbol{\epsilon}'\boldsymbol{\epsilon}$ and $\mathbf{v}_\beta\mathbf{v}_\beta'$, respectively (Dempster et al. 1981). In the E (expectation) step the expected values of these statistics are computed as follows. Let

$$\text{(A.1)} \quad \mathbf{P}^{(k)} = \mathbf{X}'\mathbf{X} + \sigma^{2(k)} \begin{bmatrix} \mathbf{O}_{2\times 2} & \mathbf{O}_{2\times 2I} \\ \mathbf{O}_{2I\times 2} & \mathbf{V}^{(k)\text{-}1} \end{bmatrix}.$$

The regression coefficients are then estimated as

$$\text{(A.2)} \quad \boldsymbol{\gamma}^{(k)} = [\boldsymbol{\mu}^{(k)\prime}, \mathbf{v}_\beta^{(k')}]' = \mathbf{P}^{(k)^{-1}}\mathbf{X}'\mathbf{y}$$

and the estimate of the variance–covariance matrix of the estimated regression coefficients, $\mathbf{V}_\gamma$, is given by

$$\text{(A.3)} \quad \mathbf{V}_\gamma^{(k)} = \begin{bmatrix} \mathbf{V}_\mu^{(k)} & \mathbf{C}_{\mu,\beta}^{(k)} \\ \mathbf{C}_{\mu,\beta}^{(k)\prime} & \mathbf{V}_\beta^{(k)} \end{bmatrix} = \sigma^{2(k)}\mathbf{P}^{(k)^{-1}}$$

where $\mathbf{V}_\mu$ and $\mathbf{V}_\beta$ denote the variance matrices of $\boldsymbol{\mu}$ and $\mathbf{v}_\beta$, respectively, and $\mathbf{C}_{\mu,\beta}$ denotes the covariance matrix between $\boldsymbol{\mu}$ and $\mathbf{v}_\beta$.

Conditional on these estimates of $\boldsymbol{\gamma}$ and $\mathbf{V}_\gamma$, the expected values of the sufficient statistics for $\sigma^2$ and $\mathbf{V}$ are

$$\text{(A.4)} \quad S_{\sigma^2} = E(\boldsymbol{\epsilon}'\boldsymbol{\epsilon} \mid \mathbf{y}, \mathbf{V}^{(k)}, \sigma^{2(k)})$$

$$= \mathbf{y}'\mathbf{y} - 2\mathbf{y}'\mathbf{X}\boldsymbol{\gamma}^{(k)} + \boldsymbol{\gamma}^{(k)\prime}\mathbf{X}'\mathbf{X}\boldsymbol{\gamma}^{(k)} + \text{tr}\,(\mathbf{X}'\mathbf{X}\mathbf{V}_\gamma^{(k)})$$

$$\text{(A.5)} \quad \mathbf{S}_V = E(\mathbf{v}_\beta\mathbf{v}_\beta' \mid \mathbf{y}, \mathbf{V}^{(k)}, \sigma^{2(k)}) = \mathbf{v}_\beta^{(k)}\mathbf{v}_\beta^{(k)\prime} + \mathbf{V}_\beta^{(k)}.$$

This completes the E step.

For the M (maximization) step, we compute the maximum likelihood estimates of $\sigma^2$ and $\mathbf{V}$. For $\sigma^2$ this is simply

$$\text{(A.6)} \quad \sigma^{2(k+1)} = S_{\sigma^2}/n.$$

The estimator for $\boldsymbol{\Sigma}$ (and hence $\mathbf{V}$) can be obtained from $\mathbf{S}_v$. Specifically

$$\text{(A.7)} \quad \boldsymbol{\Sigma}_{pq}^{(k+1)} = \sum_{i=1}^{I}(\mathbf{V}_{\gamma 2i+p,2i+q}^{(k)} + \gamma_{2i+p}^{(k)}\gamma_{2i+q}^{(k)})/I$$

for $p,q = 1,2$. Here, $\mathbf{V}_{\gamma 2i+p,2i+q}^{(k)}$ is the $2i + p, 2i + q$ element of the matrix $\mathbf{V}_\gamma$ in the $k$th iteration.

Given $\sigma^{2(k+1)}$ and $\boldsymbol{\Sigma}_{pq}^{(k+1)}$ for $k = 0$, one can compute (A.1) through (A.7) and then use the results to obtain values $\sigma^{2(k+1)}$ and $\boldsymbol{\Sigma}_{pq}^{(k+1)}$ for $k = 1$, recompute (A.1) through (A.7), etc., until adequate convergence is achieved.

# Influence of Cover on the Spatial Distribution of Littoral-zone Fishes[1]

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Littoral-zone fish are thought to reduce predation pressure by seeking refuge within macrophyte beds or near the lake bottom, or by aggregating. We used underwater video to measure the spatial aggregation of prey fishes in the littoral habitat. At low density, fish were more aggregated off the bottom in open habitats than when found off the bottom within macrophyte beds. At high densities, fish were primarily observed near the lake bottom, and the presence or absence of macrophyte beds had little influence on spatial behavior. No difference could be detected in the degree of aggregation seen in fish on the lake bottom inside or outside macrophyte beds. Our results lend field support to theoretical and laboratory research that suggests aggregative behavior in littoral-zone fish is strongly influenced by fish density and by habitat structure.

Il est suggéré par plusieurs auteurs que les poissons littoraux réduisent la pression de prédation en cherchant refuge dans les herbiers ou près du fond du lac, ou en s'agrégeant. Nous avons utilisé la vidéo pour déterminer l'hétérogénéité spatiale des poissons proies dans une variété d'habitats littoraux. À de faibles densités, les poissons seraient plus agrégés quand ils se trouvent dans la colonne d'eau dans les zones ouvertes que quand ils se trouvent dans les herbiers. À de densités élevées, les poissons se trouvaient en principe à proximité du fond du lac. La présence ou l'absence des macrophytes avait peu d'influence sur la distribution spatiale des poissons. Pas de difference dans le degré d'agrégation était observée chez les poissons se trouvant au fond du lac à l'intérieur ou à l'extérieur des herbiers. Nos analyses appuient, par la recherche in situ, la théorie qui suppose que le comportement grégaire des poissons littoraux est fortement influencé par la densité des poissons et par la structure de l'habitat.

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Random distributions of animals and plants are rare (Taylor et al. 1978). The spatial heterogeneity of animal populations is known to be density dependent (Taylor 1984; Downing et al. 1987; Folt 1987; Pinel-Alloul et al. 1988) and varies with biological and physical factors such as predation (Anscombe 1950), body size (Peters 1983), and habitat structure (Benson and Magnuson 1992). Animal populations may aggregate to acquire food, reproduce, and avoid predation. Predation is thought to increase prey aggregation because prey can diminish predation pressure by cooperative predator detection, numerical dilution, and predator confusion (Foster and Treherne 1981; Milinski 1984; Turner and Pitcher 1986). However, because prey aggregation may increase competition and intragroup aggression (Taylor and Taylor 1977; Sih 1987), prey may be less aggregated when predators are not present (Rasmussen and Downing 1988; Nelson and Bonsdorff 1990).

Characteristics of the shallow-water habitat may influence predation risk and thus alter the spatial distribution of littoral-zone fish. Several authors have suggested that littoral-zone communities exhibit temporal and spatial patterns of species abundance and distribution that are related to the presence of aquatic macrophytes, water temperature, dissolved oxygen, depth gradients, competition, and predator–prey interactions (Gilinsky 1984; Holt 1984; Keast 1984; Noble 1986; Ploskey 1986; Killgore et al. 1989; Boudreau 1992). In the littoral zone, aquatic macrophytes are thought to enhance the survival probability of prey fish by decreasing encounter rate (Hershey 1985) or predation rate (Crowder and Cooper 1982; Savino and Stein 1982; Werner et al. 1983). Small bluegill (*Lepomis macrochirus*) have been shown to be restricted to dense vegetation in the presence of piscivorous fish (Werner et al. 1983). Some adverse effects of increased vegetation structure have been documented, however. Some predators, such as northern pike (*Esox lucius*), are adapted to catching their prey among vegetation, and other predators, such as largemouth bass (*Micropterus salmoides*), may change their predatory tactics from searching to ambushing as plant density increases (Savino and Stein 1989). Bluegill predation rates have, however, been seen to decline with increasing plant density (Savino et al. 1992).

Prey may also seek cover by staying near the bottom. Savino and Stein (1989) found that 80–100% of fathead minnows (*Pimephales promelas*) generally stayed near the bottom of an experimental pool, regardless of plant density or predator type. Noakes (1992) found that young-of-the-year brook trout (*Salvelinus fontinalis*) stayed motionless under the cover of vegetation or rocks on the bottom to

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TABLE 1. Characteristics of the three study sites. "Inside" and "Outside" refer to samples taken inside and outside the macrophyte bed, respectively; $Z$ is the site depth (m) and $B$ is the mean macrophyte biomass sampled in July and August 1991 (g macrophyte dry mass$\cdot$m$^{-2}$). Dominant macrophytes were *Myriophyllum spicatum* (M), *Ceratophyllum demersum* (C), *Vallisneria americana* (Va), *Elodea canadensis* (E), and *Potamogeton epihydrus* (P). Taxonomy follows Fassett (1957). Standard deviations are given in parentheses. Sample numbers were 6 for estimates of $B$ and 48 for estimates of $Z$.

| | Site 1 | | Site 2 | | Site 3 | |
|---|---|---|---|---|---|---|
| | Inside | Outside | Inside | Outside | Inside | Outside |
| $Z$ | 3<br>(0) | 3.9<br>(0.25) | 3.5<br>(0.4) | 4<br>(0) | 2.9<br>(0.15) | 3.1<br>(0.25) |
| $B$ | 10.66<br>(1.19) | — | 1.75<br>(1.61) | — | 1.40<br>(0.00) | — |
| Dominant species | M, C, Va | — | E, P, Va | — | Va | — |
| Substrate | Sand | Sand | Gravel | Gravel | Sand | Sand |

lessen the risk of predation. Cryptic fish may also benefit by blending with the bottom (Donnelly and Dill 1984) or by flattening their bodies against the substrate (Thorpe et al. 1992). By staying near the bottom, fish can also lessen attacks from wading predators (Power 1987).

In the absence of structural cover, small fish are thought to restrict their activities to shallow habitats (De Vries 1990) and form aggregations that provide protection from predation (Seghers 1974; Pitcher 1986; Morgan and Colgan 1987; Magurran 1990). Small perch (*Perca fluviatilis*) have been shown to disperse from schools when moving into vegetation to feed (Mikheev 1985). Facultative schooling by small bluegill within vegetation may provide an additional measure of protection (Mittelbach 1984). Savino and Stein (1989) found that 50% of young-of-the-year bluegill shoaled in the absence of predators such as largemouth bass and pike, while 50–80% shoaled in the presence of predators at low plant densities and less than 20% at medium and high densities. De Vries (1990) observed that juvenile bluegill remained dispersed at low largemouth bass activity, while at higher predator activities, bluegill were almost entirely schooled. The size and cohesion of fish groups are thought to be determined by conflicting demands imposed by predation risk, which promotes aggregation, and the pressure to compete for food, which promotes dispersion (Seghers 1981; Magurran and Pitcher 1983). Aggregations of fishes have been observed to be formed not only for protection but for feeding purposes. Schools of plankton-eating fishes forage on small-scale clumps of zooplankton, presumably greatly increasing their foraging efficiency (McNaught and Hasler 1961).

Empirical studies describe a general relationship between density and spatial variance for larval fish (Cyr et al. 1992). The results of laboratory and seminatural field experiments indicate that habitat structure and predation risk may alter prey spatial aggregation (Butler 1989; Benson and Magnuson 1992). Unfortunately, however, small-scale (1–10 m) heterogeneity has not been documented for fish in natural lakes, because of the difficulty of measuring underwater spatial heterogeneity (Steele 1978).

The objective of this study is to find how spatial heterogeneity in littoral-zone fish varies with density and habitat structure such as macrophyte cover and position in the water column. Following theory, we postulate that fish should be more aggregated in open habitats or above the bottom than they are when found near to the bottom of lakes or in macrophyte beds where they are less exposed to predation.

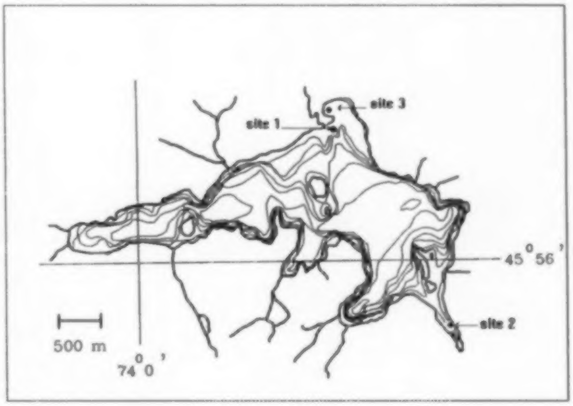

FIG. 1. Location of study sites in Lac de l'Achigan, Québec.

## Methods

### Site Description

This study was carried out in the littoral zone of Lac de l'Achigan, Québec, Canada (45°56'N, 74°W), a 5-km$^2$, soft-water (alkalinity 11.5 mg CaCO$_3$$\cdot$L$^{-1}$), oligotrophic lake (total phosphorus 6.4 $\mu$g$\cdot$L$^{-1}$; Lamontagne and Gauthier 1974). Littoral fish communities that we observed consisted mainly of small largemouth bass (<100 mm), pumpkinseed (*Lepomis gibbosus*), yellow perch (*Perca flavescens*), and bluntnose minnow (*Pimephales notatus*), although there are some predaceous largemouth bass >30 cm in length in the lake. Three sites were sampled in the littoral zone of the lake in the summer of 1991 (Fig. 1). Each site included open lake bottom and macrophyte patches. Site depths were measured and macrophyte density was estimated by measuring plant standing biomass ($B$, dry mass per square metre; Downing and Anderson 1985). Macrophyte density in Lac de

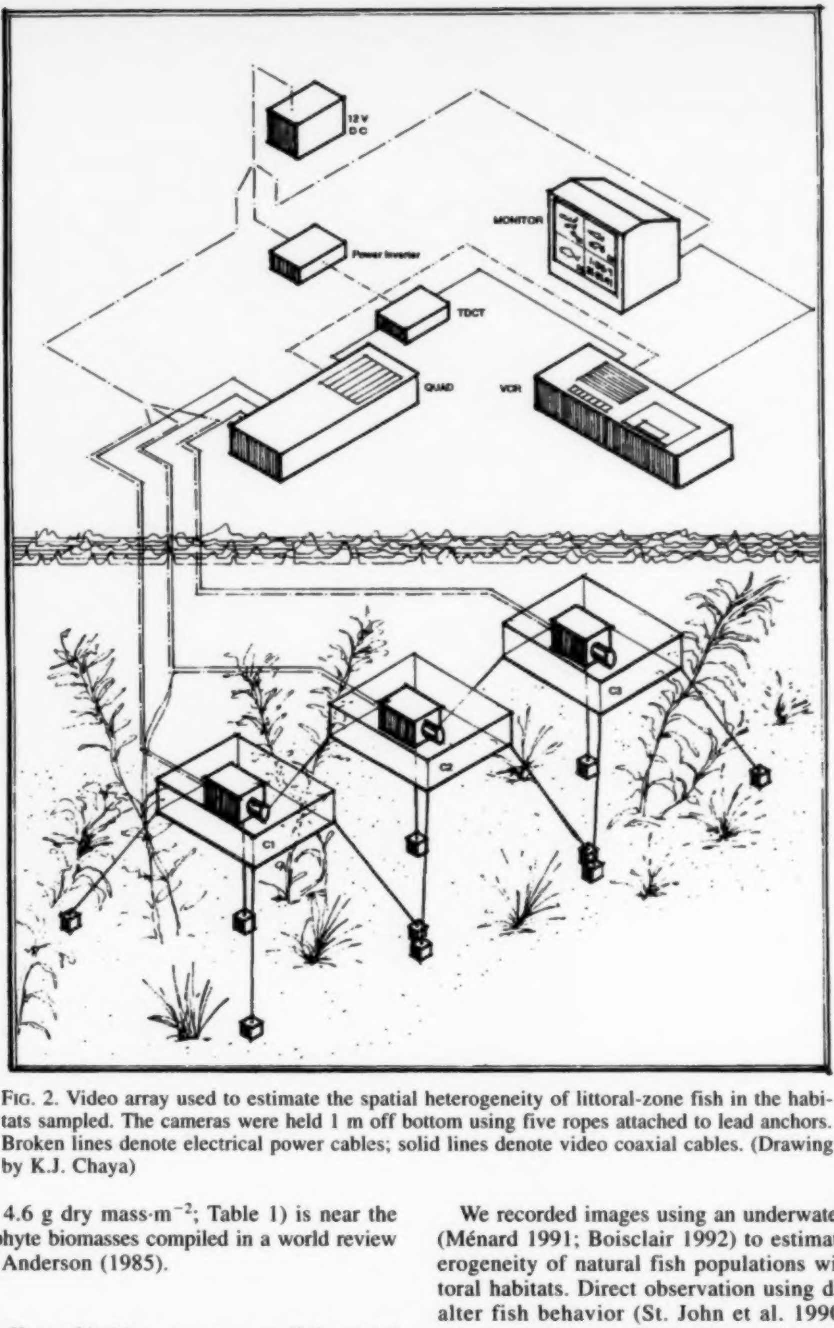

FIG. 2. Video array used to estimate the spatial heterogeneity of littoral-zone fish in the habitats sampled. The cameras were held 1 m off bottom using five ropes attached to lead anchors. Broken lines denote electrical power cables; solid lines denote video coaxial cables. (Drawing by K.J. Chaya)

l'Achigan (mean 4.6 g dry mass·m$^{-2}$; Table 1) is near the average of macrophyte biomasses compiled in a world review by Downing and Anderson (1985).

### Field Methods

To assess the effect of habitat structure on fish spatial distribution and to insure independence among observations, we followed a three-factor (2 habitats × 2 depths × 2 times of day) randomized nested design at each of the three sites, corresponding to 24 site–habitat–depth–time combinations. The order in which the sites and habitat types were sampled during the month of July was assigned at random with a pseudo-number random generator. In August, the experiment was repeated in exactly the same fashion, yielding 48 site–habitat–depth–time–date combinations in all.

We recorded images using an underwater video technique (Ménard 1991; Boisclair 1992) to estimate the spatial heterogeneity of natural fish populations within different littoral habitats. Direct observation using divers is known to alter fish behavior (St. John et al. 1990; Graham 1992) whereas video recording allows a less intrusive and continuous record of fish behavior to be made (Michalopoulos et al. 1992).

The video system (Fig. 2) consists of three surveillance cameras (Panasonic™ models WV-CD22 and WV-BL 202) enclosed in waterproof Plexiglas™ housings, placed at randomly chosen points within the habitat. The cameras were connected to a video-processor (Panasonic™ Color QUAD System; WJ-450) allowing the simultaneous recording and display of three images. A TDCT (time and date camera

TABLE 2. Results of multiple regression analysis of the influence of macrophyte beds and water column position on the spatial aggregation of littoral fish communities in Lac de l'Achigan. Analyses were performed following eq. 1 using $\log_{10}(M)$ as a covariable and presence or absence of macrophytes ($H$) and proximity to bottom ($D$) as dummy variables. $H = 0$ = outside macrophyte beds, $H = 1$ = inside macrophyte beds; $D = 0$ = lake bottom, $D = 1$ = off bottom. Interaction terms test for difference in slope of the relationship between $\log_{10}s^2$ and $\log_{10}M$; partial $t$-statistic and associated probability ($p$) indicate the partial significance of each variable in the prediction of $\log_{10}(s^2)$. Analyses were performed by entering the full model (eq. 1) in a multiple regression analysis and using backwards elimination (Hocking 1976) to progressively remove nonsignificant variables.

| Variables | Regression coefficient | Partial $t$ | $p$ |
|---|---|---|---|
| Intercept | −1.40 | −3.03 | 0.004 |
| $\log_{10}(M)$ | 2.40 | 11.4 | <0.001 |
| $D$ | 1.69 | 3.41 | <0.001 |
| $\log_{10}(M)\cdot D$ | −1.28 | −3.88 | <0.001 |
| $(H)\cdot(D)$ | −0.699 | −2.47 | 0.018 |
| $\log_{10}(M)\cdot H\cdot D$ | 0.780 | 2.55 | 0.015 |

$R^2 = 0.94$, $n = 47$, $F = 136.68$, $p < 0.001$

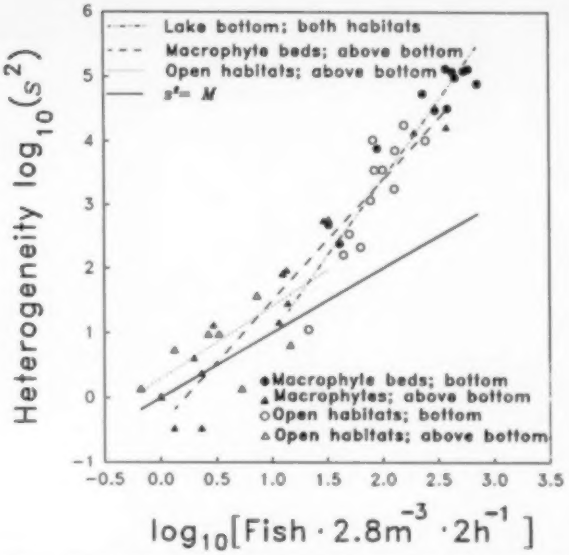

FIG. 3. Predicted relationship (Table 2) between ($s^2$) and ($M$) for fish heterogeneity data from different habitats. Symbols indicate observed spatial variances, and broken lines are the fitted relationships taken from Table 2 and eqs. 2–4. The solid line represents the variance that would have been found if the fish were distributed randomly in space.

titeler) was used to time–date stamp images, and images were recorded using an 8-mm VCR (Sony™ EV-S1) monitored with a small black and white television (Panasonic TRG-513CT). One observation site was filmed at a time by the three cameras for 2 consecutive h. All combinations of sites, habitats, depths, and times of day were considered separate treatments and were filmed in random order on separate days. All species of fish were present at all sampling times in all habitats.

### Image Analysis

Given the water transparency in Lac de l'Achigan, each camera filmed approximately 2.8 m$^3$ of water (Boisclair 1992). When the cameras were placed on the bottom, the field of view was reduced to 1.44 m$^3$, so the mean fish density was corrected and was expressed as number of fish per 2.8 m$^3$ for all samples (Aboul Hosn 1993). We analyzed each videotape and recorded the cumulative number of fish seen by each of the three cameras over the full 2 h of filming. We thus obtained three estimates of the density of fish at each sampling site, estimated at three distinct places by the three cameras. The average of the cumulative number of fish seen by each camera ($M$) and the variance associated with the three replicate images in each habitat ($s^2$) were calculated. Comparisons of spatial aggregation among sites were made by comparing the degree of variation among samples ($s^2$) observed at comparable values of $M$. A site showing low spatial heterogeneity would have lower $s^2$ for a given $M$ relative to a site with higher spatial heterogeneity (Downing 1991).

### Statistical Analysis

Variations of fish numbers viewed by the different cameras yield direct estimates of the spatial variability of fish abundance. Our hypotheses were tested simultaneously using a multiple regression model with interaction terms:

$$\begin{aligned}(1)\quad \log_{10}s^2 &= \beta_0 + \beta_1\log_{10}(M) + \beta_2(H) + \beta_3(D)\\ &\quad + \beta_4\log_{10}(M)\cdot(H) + \beta_5\log_{10}(M)\cdot(D)\\ &\quad + \beta_6\log_{10}(H)\cdot(D) + \beta_7\log_{10}(M)\cdot(H)\cdot(D) + \epsilon\end{aligned}$$

where $\log_{10} s^2$ is the dependent variable, $H$ is the coded habitat (0 = outside macrophyte beds, 1 = inside macrophyte beds), $D$ is the coded depth (0 = lake bottom, 1 = off bottom), and $\beta_0$–$\beta_7$ are fitted constants. The model was fitted to the data (Neter et al. 1990; see also Pinel-Alloul et al. 1988; Rasmussen and Downing 1988). The mean density must be included as a covariable because the spatial $s^2$ that would indicate randomness varies directly with $M$ (Downing 1991). Interactions among the variables were considered because $s^2$ may rise at different rates with $M$ in different treatments. The significance of the effect of each of the variables on spatial heterogeneity was judged by the significance of the partial $t$-value associated with each regression coefficient. Departure from randomness ($s^2 = M$) was used as an estimate of the relative degree of spatial aggregation and was determined by comparison of the degree of variation among samples observed at comparable values of $M$ (Downing 1991). Differences in responses among sites were sought by examining the residuals of eq. 1, using the Fisher–Snedecor test (Draper and Smith 1981).

## Results and Discussion

In the ANCOVA of eq. 1 (Table 2), the significant $t$-values ($p < 0.05$) associated with the regression coefficients demonstrated several effects in addition to the highly significant effect of $M$ on $s^2$. Depth, the interactions between fish den-

TABLE 3. Relative number of fish in each of the habitats. Average fish densities are calculated across all sampling sites and dates (Aboul Hosn 1993).

| Habitat type | Mean fish·2.8 $m^{-3}$·2 $h^{-1}$ | % |
|---|---|---|
| Macrophyte/bottom | 251 | 81 |
| Macrophyte/above bottom | 11 | 4 |
| Open habitats/bottom | 43 | 14 |
| Open habitats/above bottom | 3 | 1 |

TABLE 4. Analysis of the residuals testing for differences in spatial aggregation among sampling sites (Fig. 1). ANOVA was performed on the residuals of eq. 1 (Table 2). The Fisher–Snedecor test ($F$) shows whether mean residuals are significantly different among sites.

| Group | Mean residual | $F$-values | $p$ |
|---|---|---|---|
| Macrophyte beds | 0.048 | 0.31 | 0.74 |
| Open habitats | 0.226 | 0.98 | 0.39 |

sity and depth and between habitat and depth, as well as the three-way interaction of density, habitat, and depth all had significant effects on the spatial heterogeneity of littoral fish. When fish were found near the lake bottom, there was no statistically significant influence of presence or absence of macrophyte cover on the level of aggregation found in these natural fish communities.

Substituting the regression coefficients in Table 2 for $\beta_0$–$\beta_7$ in eq. 1 and simplifying allows the calculation of spatial variance functions for each habitat combination. Because $D = 0$ for fish found near the bottom, the spatial variance of fish found in that habitat varies as

$$(2)\quad s^2 = 0.0398M^{2.40}.$$

The significant interaction between abundance, habitat, and depth (Table 2) means that $s^2$ rises most rapidly with $M$ near the bottom in both habitats. Above the bottom, within macrophyte beds ($D = 1$, $H = 1$), eq. 1 simplifies to

$$(3)\quad s^2 = 0.0387M^{1.9}$$

whereas above the bottom, outside the macrophyte beds ($D = 1$, $H = 0$), eq. 1 simplifies to

$$(4)\quad s^2 = 1.93M^{1.12}$$

meaning that spatial aggregation rises least rapidly with fish density ($M$) outside the macrophyte beds.

These analyses allow us to detect the influence of density and habitat on spatial heterogeneity in littoral fishes. The degree of aggregation increases as the data points lie further above the line representing random distribution ($s^2 = M$) (Fig. 3). The most striking departure of these response functions from randomness was seen at high densities, suggesting that fish are most aggregated when densities are high.

High densities of fish and thus greatest degree of aggregation were primarily observed near the lake bottom, and no difference could be detected in the degree of aggregation seen in fish on the lake bottom inside or outside the macrophyte beds (Fig. 3). This suggests that at high densities, on the bottom of lakes, the presence or absence of macrophyte beds has little influence on spatial behavior.

At low densities (i.e., log $M < 1.3$), however, the effect of macrophyte beds on spatial heterogeneity can be significant. Fish densities <1.3 were usually observed in both the open habitats and macrophyte beds when fish were off the bottom. At low densities, fish tended to be most aggregated when swimming off the bottom in open habitats but were nearly randomly distributed when found swimming off the bottom within macrophyte beds (Table 2; Fig. 3). It thus appears that being within macrophyte beds decreases aggregative behavior. At low densities, fish may effectively reduce predation by aggregating in open habitats above the bottom, while the upper levels of macrophyte beds may afford some protection against predation, reducing the necessity of aggregative behavior. Macrophyte cover had a significant effect on spatial heterogeneity when fish were swimming off the bottom. Thus, the potential importance of macrophyte presence or absence appears to vary with fish density and their location in the water column.

If approximately equal littoral areas are occupied by macrophyte beds and open bottoms, and our observations are an unbiased record of fish activities, a provisional time budget suggests that fish spend more than 80% of their time in macrophyte beds and nine times more time is spent near the bottom than in upper waters (Table 3). These calculations agree with those of Chapman and Mackay (1984), who found that 84% of small northern pike were observed in vegetated areas and 16% in nonvegetated areas. Naturally, if more than 50% of the littoral zone is occupied by macrophyte beds, fish may spend an insignificant fraction of their time in open areas. Although our calculated time budgets are crude, it appears that littoral-zone fish spend most of their time at the bottom of macrophyte beds. Since quantitative measures of littoral habitat use are rare, and because the influence of littoral habitat composition on time allocation is difficult to quantify, we do not, as yet, know how general this result may be. Future studies documenting the influence of habitat structure on fish time allocation would add significantly to our knowledge.

We observed similar responses at all the sites that we sampled. Analysis of the residuals of eq. 1 (Table 4) shows no significant ($p > 0.05$) differences in the residual spatial variance of fish communities at the three sampling sites. The spatial behavior of littoral-zone fish does not, therefore, appear to be site specific, at least in this lake.

Our results agree with the hypothesis that prey are less aggregated at sites where predation pressure is presumably low. At low densities, fish in open habitats above the bottom are consistently aggregated. At these low densities, fish in macrophytes above the bottom are nearly randomly distributed. Previous studies suggest that fish would be more exposed to predation above the bottom in habitats without macrophytes. If aggregation is a predator-avoidance response, we can conclude that fish off the bottom may decrease aggregation when they are found within macrophyte beds.

Such flexibility in fish behavior is important when predation risk is variable. Fish are known to constantly reassess their environment and adjust their behavior accordingly (Magurran 1990).

Although experimental laboratory studies have shown that the spatial distribution of prey fishes is influenced by habitat structure and predation pressure, quantitative in situ experiments are essential to compare experimental results with those found in natural ecosystems. Our study provides the first field evidence that at low densities, fish are least aggregated in habitats that offer the greatest potential for protection against predation.

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# Evaluation of the Depensatory Fishing Hypothesis as an Explanation for Population Cycles in Fraser River Sockeye Salmon (*Oncorhynchus nerka*)

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Causes of cyclic fluctuations in the abundance of Fraser River sockeye (*Oncorhynchus nerka*) have been the focus of considerable debate for much of this century. Most hypotheses to explain cyclic behaviour in Fraser River sockeye centre on identifying agents of depensatory mortality, and several recent papers argue that sockeye cycles are maintained primarily by depensatory fishing patterns. We assess the evidence for depensatory fishing as an explanation for these cycles. Using simulations, we demonstrate that empirical evidence for depensatory fishing is likely an artifact caused by bias in estimating stock composition of catches in mixed-stock fisheries. Moreover, recent trends in harvest rates among comigrating stocks with asynchronous cycles are inconsistent with the depensatory fishing hypothesis. It also seems very unlikely that aboriginal and early commercial fisheries were intensive enough to maintain cycles that persisted prior to 1860. Only during the unregulated, early commercial fishery prior to the collapse of the upriver runs following the 1913–14 disaster at Hell's Gate is there any convincing evidence of depensatory fishing.

Les causes des fluctuations cycliques de l'abondance des saumons rouges (*Oncorhynchus nerka*) dans le fleuve Fraser ont été le sujet de débats passionnés au cours d'une bonne partie du vingtième siècle. La plupart des hypothèses qui tentent d'expliquer le comportement cyclique du saumon rouge dans le fleuve Fraser se greffent autour de l'identification d'agents de mortalité anticompensatoire, et plusieurs articles récents soutiennent que les cycles du saumon rouge sont maintenus principalement par des pratiques de pêche anticompensatoire. Nous avons étudié les données concernant la pêche anticompensatoire pour déterminer si ce type de pêche pouvait expliquer ces cycles. À l'aide de simulations, nous avons démontré que les preuves empiriques de pêche compensatoire résultent probablement d'un artefact causé par des erreurs dans le calcul de la composition des stocks de poissons capturés dans le cadre de la pêche mixte. En outre, les tendances récentes dans les taux de capture chez les stocks de poisson à cycle asynchrone migrant ensemble ne cadrent pas avec l'hypothèse concernant la pêche anticompensatoire. Il semble également très improbable que la pêche autochtone et la pêche commerciale à ses débuts aient été si importantes pour maintenir des cycles qui ont persisté avant 1860. Ce n'est que durant la période non réglementée correspondant aux débuts de la pêche commerciale, avant la chute de la montaison des saumons à la suite du désastre de Hell's Gate en 1913–1914, que les données indiquaient de façon convaincante qu'il y a eu pêche anticompensatoire.

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Cyclic fluctuations in abundance are characteristic of many fish populations with single or dominant reproductive age classes (Townsend 1989). Perhaps the most dramatic examples of population cycles in fishes are seen in Pacific pink (*Oncorhynchus gorbuscha*) and sockeye salmon (*O. nerka*). Pacific pink salmon invariably mature and die at age 2 and persistent 2-yr cycles occur in many populations. Similarly, most Fraser River sockeye salmon mature and die at age 4 and most populations inhabiting the largest lakes in the Fraser River drainage exhibit persistent 4-yr cycles. In this paper, we assess the evidence for depensatory fishing as an explanation for these cycles in Fraser River sockeye salmon.

## Occurrence and Pattern of Cycles

It is important to recognize that fluctuations or "cycles" in abundance vary among Fraser River sockeye populations both in pattern and persistence. Of approximately 20 sockeye populations in the Fraser River watershed that are enumerated routinely, eight exhibit persistent 4-yr cycles with a predictable dominant-year cycle line every 4 yr. In this paper, a cycle line refers to a particular sequence of years in the 4-yr interval that comprises consecutive generations. For example, the 1990 cycle line refers to the sequence of years 1990, 1986, 1982, ..., 1950, etc. The dominant-year cycle line refers to the sequence of years wherein the run size is persistently larger than the other cycle lines. Run size is persistently lowest on off-year cycle lines and intermediate on the subdominant cycle line.

The lower Adams River and lower Shuswap River spawning populations cycle in a dominant–subdominant–low–low (DSLL) cyclic pattern. The persistent DSLL pattern in these populations has been the focus of the "cyclic dominance" debate (Ricker 1950; Ward and Larkin 1964; Larkin 1971; Collie and Walters 1987; Walters and Staley 1987; Welch and Noakes 1990). The term "cyclic dominance" refers to the

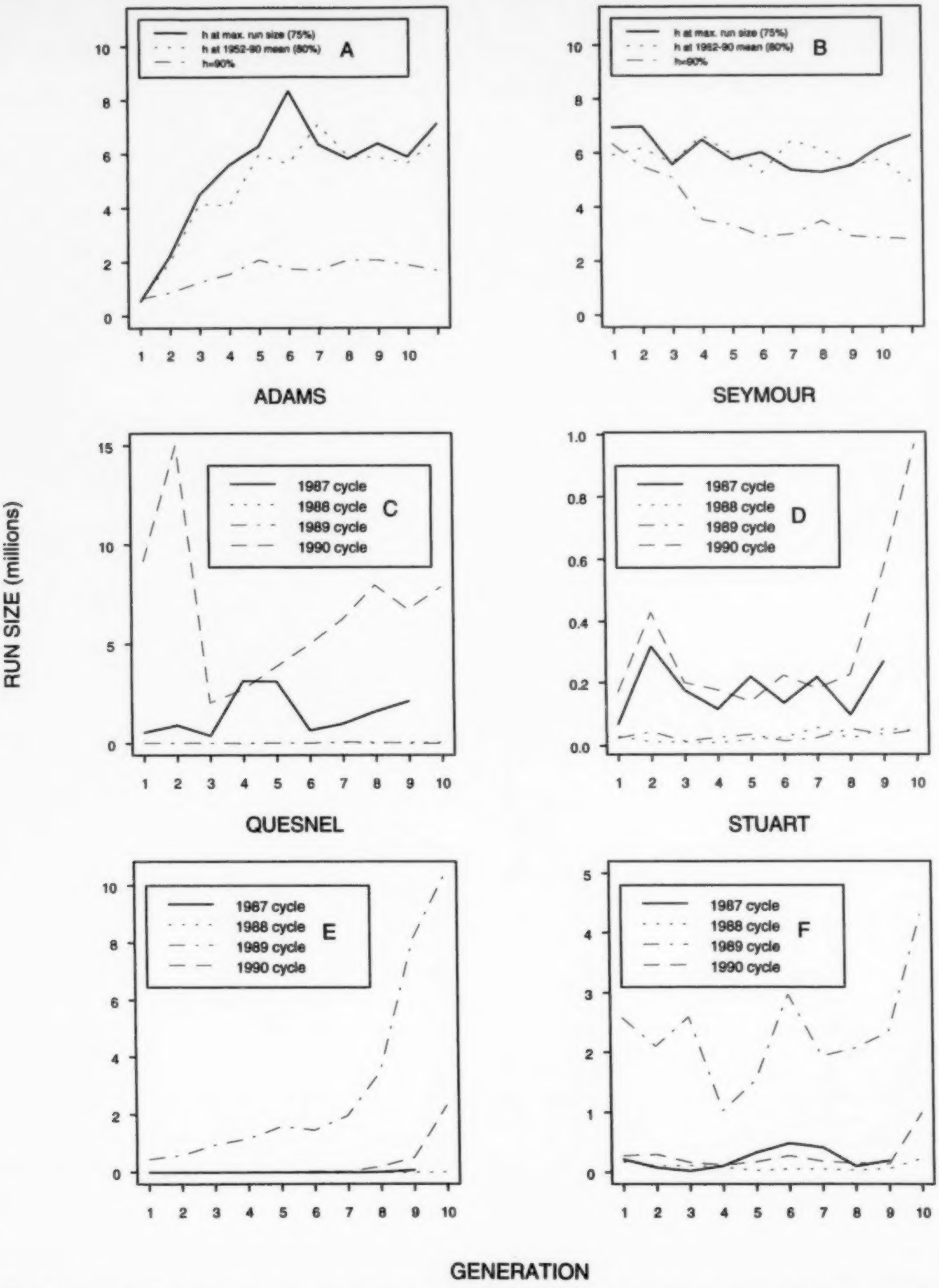

FIG. 1. Trends in run size by generation (i.e., cycle line) for (A) a simulated population following a single large catastrophic reduction in run size, (B) a simulated population without a catastrophic reduction in run size, and (C–F) cyclic populations of Fraser River sockeye (historical (nominal) estimates, 1952–90). Simulated trends in run size are the mean of 500 trials for cycle lines regulated by a stochastic Ricker stock–recruitment function fished at sustained harvest rates (*h*) of 75, 80, and 90% (see Appendix).

hypothesis that intrinsic interactions among year classes lead to differences in biological productivity or equilibrium stock size among cycle lines. Populations that rear in Quesnel Lake and the largest runs to the Stuart–Takla–Trembleur lakes region (early Stuart Lake and late Stuart Lake runs) have cycled in a DLLL pattern for most of the last 10 generations but have recently tended toward a DSLL pattern. Seton Lake sockeye (Gates and Portage creeks) exhibit persistent 4-yr cycles with a DSLS pattern. These two runs have asynchronous cycles with the dominant cycle lines offset by 2 yr. Seymour River sockeye have fluctuated with two persistent off-years and two dominant years.

Other populations have fluctuated with an apparent 4-yr "cycle" in abundance but have not exhibited persistent cyclic patterns. These include Chilko River, Stellako River, Nadina River, Bowron River, Raft River, and Cultus Lake sockeye (Cass 1989). Chilko River sockeye historically have fluctuated with an irregular pattern of two adjacent off years and two dominant years. Year-class abundance of Stellako River sockeye has fluctuated in an irregular 4-yr pattern with no persistent dominant line since 1952. Nadina River sockeye cycled in a DSLL pattern in the 1950s but have recently fluctuated with a 2-yr pattern. Particularly abundant year classes during recent declines in Bowron Lake and Raft River sockeye abundance suggest 4-yr cycles, but these populations have otherwise fluctuated without a detectable 4-yr pattern since routine escapement enumeration began. Cultus Lake sockeye have fluctuated irregularly but with a persistent dominant line since the 1950s.

Populations lacking discernable dominant-year cycle lines include Weaver Creek, Birkenhead River, and upper Pitt River sockeye. With the exception of Weaver Creek sockeye, these populations are not typical of other cyclic populations because they have a higher proportion of multiple reproductive age classes.

### Persistence of Cycles

The simplest explanation for cyclic fluctuations in abundance is that they are merely echoes of infrequent random catastrophic perturbations that persist because of the simple age structure of these populations. However, there is strong evidence that this is not the case for Fraser River sockeye. Simulation studies and historical trends both indicate that the reproductive capacity of sockeye is sufficient for sockeye populations to rebuild quickly from small spawning escapements (Ward and Larkin 1964; Walters and Staley 1987; Collie et al. 1990). The current dominant cycle line escapements of the Adams River population increased 15-fold in one generation from only 20 000 spawners in 1922 to 300 000 in 1926 (Ward and Larkin 1964) and 128-fold in five generations to 2 568 000 by 1942 (Larkin 1971). Simulations using a simple stochastic Ricker recruitment function indicate that the time required by Fraser River sockeye to rebuild following a large negative perturbation is greatly affected by prevailing harvest rates (see Appendix). With sustained harvest rates of 80%, near the historical average for cyclic populations, rebuilding and recovery of year classes decimated by catastrophe would, on average, be much quicker than has been observed for the off-year cycle lines in cyclic populations (Fig. 1). Sustained harvest rates of 90% will hold these populations at equilibrium well below the historical abundance of dominant-year cycle lines. However, harvest rates of this magnitude would reduce *all* cycle lines including dominant cycle lines to low equilibrium stock sizes thereby eliminating cycles altogether (Fig. 1 and 2).

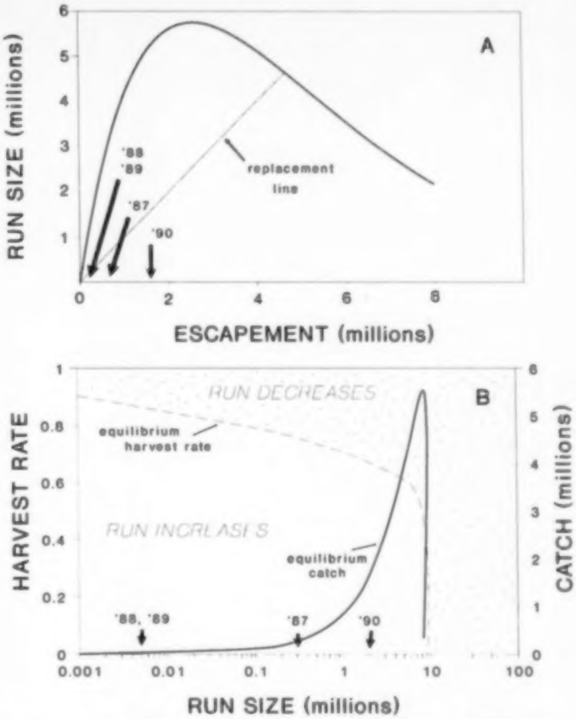

FIG. 2. (A) Ricker recruitment function for Adams River sockeye. Arrows denote the average spawning escapement by cycle line, 1952–90. (B) Equilibrium harvest rates and equilibrium catch as a function of run size (log scale) for Adams River sockeye. Arrows denote average run size by cycle line, 1952–90. (Ricker function parameters from Cass 1989.)

Frequency distributions of run size in simulated populations following perturbation and a recovery period of 6–10 generations bear little resemblance to the empirical frequency distributions for cyclic populations (Fig. 3, see Appendix for details). These simulations mimicked the length of the historical record for Fraser River populations and included environmental noise typical of Pacific salmon and sustained harvest rates of 80 or 90%. After 6–10 generations, run size distributions for perturbed (simulated) populations fished at sustained harvest rates of 80% during the recovery period deviated little from those expected from optimal escapement in unperturbed populations, irrespective of noise level. Even with a sustained harvest rate of 90% and high environmental noise (the scenario most likely to generate extreme variation in run size), the frequency distribution of run size was unimodal and differed significantly from the empirical distributions (*G*-test; $p < 0.05$). Thus, it is difficult to understand how cycles created by rare catastrophic events can be maintained by high sustained harvest rates alone. The fact that cycles *are* maintained forces us to conclude that some mechanism, either natural or man-induced, has acted to maintain cycles in these populations.

### Fishing Hypothesis

A variety of hypotheses have been proposed to account for

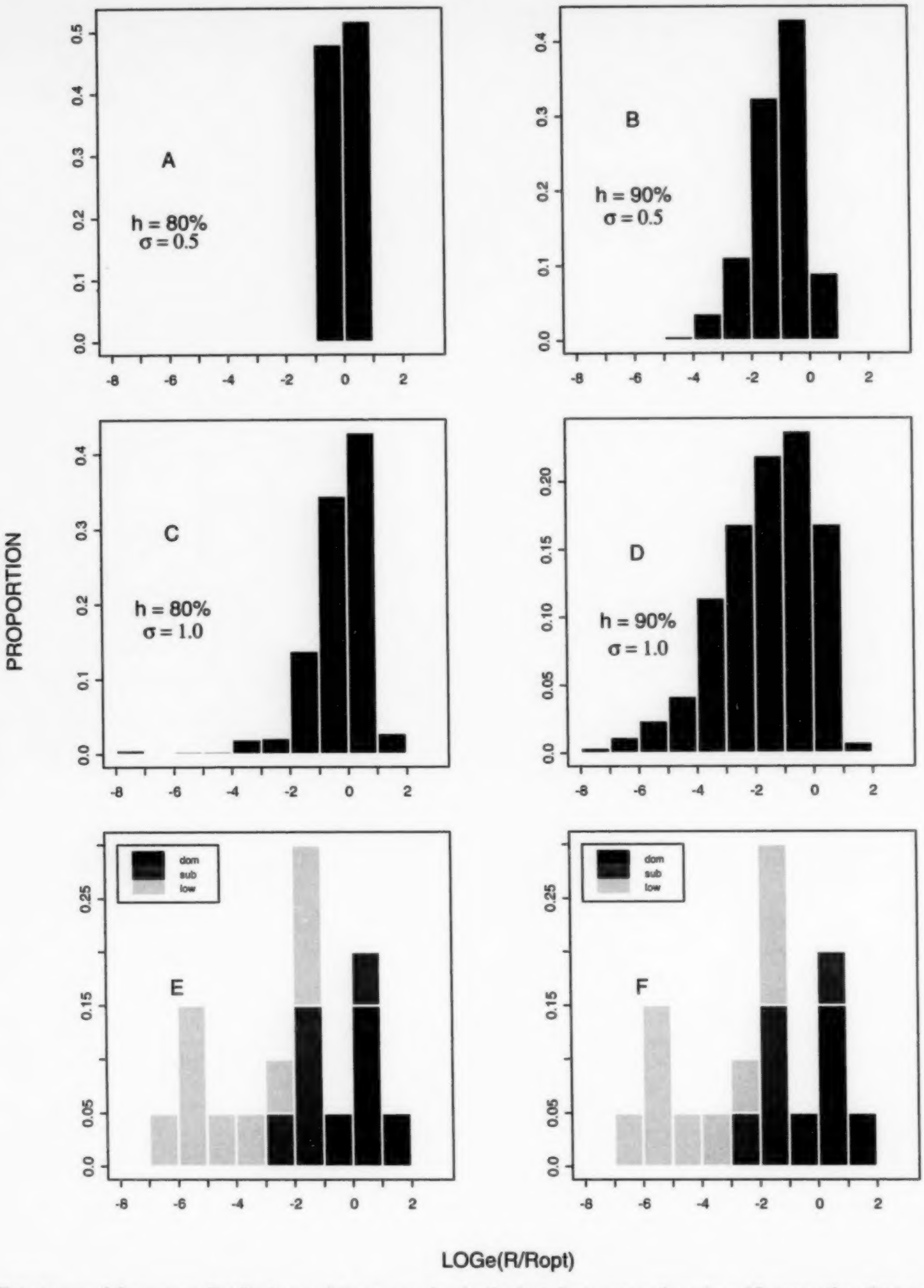

FIG. 3. Histograms of frequency distributions of mean run size in the last three generations in a 10-generation time series (with rescaling to facilitate comparison). (A–D) Simulated populations using various combinations of environmental noise ($\sigma$ = 0.5 or 1.0) and sustained harvest rates ($h$ = 80 or 90%) following a single large reduction in run size; (E and F) corresponding empirical data for cyclic populations of Fraser River sockeye shaded by cycle line (see Appendix). Both the simulated and observed data are log transformations of run size ($R$) divided by a stock-specific rescaling factor, $R_{\text{opt}}$ (the run size generating maximum sustained yield). Simulated distributions of run size are derived from 500 trials using a stochastic Ricker stock–recruitment function.

the persistence of cycles in Fraser River sockeye abundance (see reviews by Ricker 1950; Ward and Larkin 1964; Levy and Wood 1992). Several recent papers argue that sockeye cycles are maintained primarily by depensatory fishing patterns, hereafter called the "fishing hypothesis" (Collie and Walters 1987; Eggers and Rogers 1987; Walters and Staley 1987; Collie et al. 1990). Fishing mortality will be depensatory whenever a population is harvested at a proportionately higher rate in years of low abundance than in years of high abundance, as, for example, would happen if the catch each year tended to be constant. In other words, the fishing hypothesis suggests that off-year cycle lines have been overfished consistently enough to prevent them from rebuilding to levels typical of the dominant-year lines. If this is true, the economic losses associated with such overfishing are enormous (Walters and Staley 1987; Welch and Noakes 1991).

The fishing hypothesis has attracted much support because it is simple, intuitively appealing, and can explain many historic trends in abundance without postulating more complex, natural causes. We first examine evidence supporting the fishing hypothesis and then examine the following three objections to it: (1) evidence for the fishing hypothesis is potentially an artifact caused by biases in estimating the stock composition of catches in mixed-stock fisheries, (2) the fishing hypothesis does not account for the asynchrony in cycles of several upper Fraser River sockeye populations that has persisted since the 1940s, and (3) it is unlikely that aboriginal fisheries and early commercial fisheries were sufficiently intense to maintain the cyclic pattern that existed prior to 1860, let alone create it.

## Evidence Supporting the Fishing Hypothesis

Simulation studies show that management policies or fishing patterns that exert depensatory mortality will generate population cycles in Fraser River sockeye (Walters and Staley 1987). Collie and Walters (1987) presented empirical evidence for the existence of depensatory fishing mortality for Adams River sockeye based on the relationship between historical (nominal) estimates of harvest rates and run size. The relationship is presented in Fig. 4 along with similar evidence for other cyclic populations of Fraser River sockeye. Here, Adams River sockeye are pooled with other late Shuswap Lake populations to form a single stock. The inverse relationship between harvest rate and the natural logarithm of run size for the late Shuswap Lake stock is significant ($p < 0.05$), with harvest rates for the two off cycle lines higher than harvest rates for the dominant and subdominant lines, thus supporting the fishing hypothesis.

Importantly, three of the four years of lowest harvest rates reported for late-run Shuswap Lake sockeye correspond to a sequence of years in the dominant cycle line (1962–70) during which harvest rates were deliberately reduced to rebuild the dominant run following a collapse in 1962 of the returns from the 1958 spawning. In 1963, the year of the third lowest reported harvest rate, the fishing season was shortened by an industry strike and harvest rates were anomalously low in many other stocks. These four years impart the high degree of depensation in the harvest rate – run size regression (Fig. 4). For all other cyclic populations, it is not possible to reject the null hypothesis that harvest rates are constant ($p > 0.05$). The harvest rate – run size regression for Quesnel Lake and late Stuart Lake populations is influenced by outliers that dampen the statistical relationship. Although we know of no reason to exclude particular years, most dominant years for Quesnel Lake and late Stuart Lake populations are associated with lower nominal harvest rates than in the off years.

## Evidence Against the Fishing Hypothesis

### Potential Bias from Errors in Stock Identification

*Source of bias*

Estimates of run size (catch + escapement) and harvest rates (catch/run size) for individual Fraser River sockeye populations are difficult to measure. Historically, most of the catch of Fraser River sockeye has been assigned to individual populations based on stock composition analysis of catch samples using scale patterns (Woodey 1987). Misclassification of catches in mixed-stock fisheries will typically result in catches of small runs being overestimated and those of large runs being underestimated (e.g., Beacham et al. 1985a, 1985b; Wood et al. 1987). This in turn will result in harvest rates being overestimated in off years. The bias may also be exaggerated by errors in escapement estimates. Escapements in years of low abundance are potentially underestimated owing to reduced enumeration effort. Escapements in years of high returns, when escapements are estimated using mark–recapture experiments, are potentially overestimated (Symons and Waldichuk 1984).

The amount of bias in stock composition analysis depends on which stocks and how many stocks are considered in the analysis, on sample sizes, and especially on the uniqueness of the stock identification characters. Unlike genetic characters, the uniqueness of scale characters can vary considerably from year to year (Wood et al. 1989) especially for Fraser River sockeye populations where juvenile density in nursery lakes can vary enormously among cycle lines.

*Historical procedures*

Estimates of catch by stock in mixed-stock fisheries were derived historically mainly from differences in scale growth characters, principally circuli counts in the first two freshwater growth zones (Clutter and Whitesel 1956). Until the mid-1980s, estimates of stock composition were based on a graphical technique wherein the distributions of circuli counts from discrete populations, as measured from spawning ground samples, were superimposed on the distribution of mixed-stock fishery samples (Henry 1961). After the mid-1980s, more rigorous computer-based techniques have been used to estimate catch by stock (see Cook and Lord 1978).

The existence of different run timing distributions of Fraser River sockeye has long been recognized (Killick 1955; Verhoeven and Davidoff 1962). Historically, reference stocks for stock composition analysis of mixed-stock fisheries were selected by identifying those stocks expected in particular run timing groups. This procedure increased the potential for differentiating stocks by reducing the number of stocks included in the analysis and simplifying the mixture problem. Consequently, only those stocks considered to be present in a particular run timing group could be detected in a mixed-stock fishery sample. In reality, run timing is a continuous function where the abundance of an individual stock builds and falls during its migration through various fisheries. The relative abundance of any two stocks in a particular

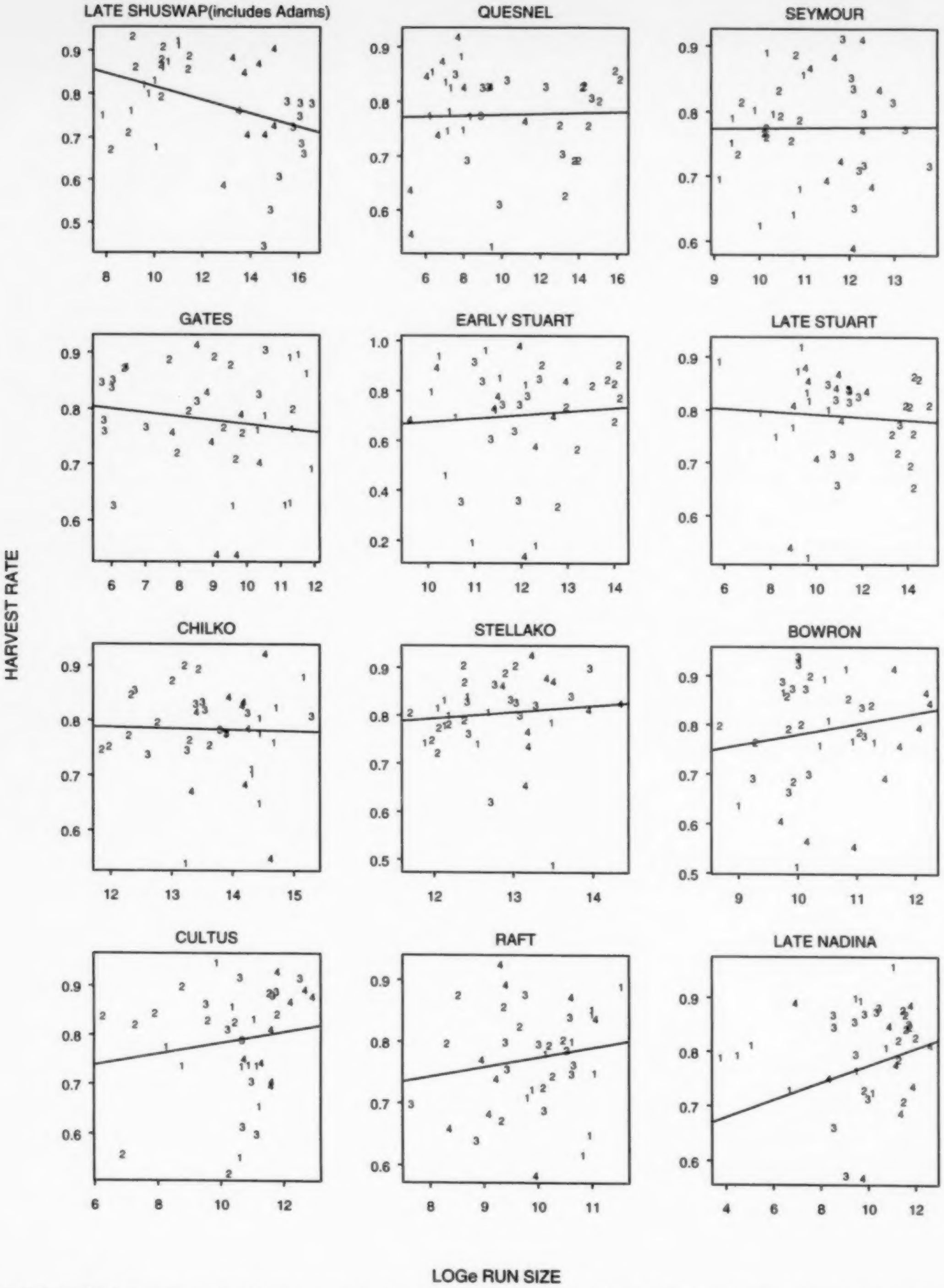

FIG. 4. Relationship between nominal harvest rate and nominal run size data for populations of Fraser River sockeye (1952–90). Numbered labels (1–4) denote harvest – run size data points for each of the four cycle lines where 1–4 indicate cycle lines 1984, 1985, 1986, and 1987.

fishery depends on both their respective run timing distributions and their total abundance. Moreover, there is considerable uncertainty about the degree of overlap in stock-specific run timing curves from year to year. This is evident in the exploitation rates sustained by the 1985-cycle lower Adams River run (a late-timed stock) during the

TABLE 1. Fraser River sockeye stocks classified as early summer, midsummer and late summer run timing groups that qualified for inclusion (sample size ≥30 fish) in simulations to reconstruct stock identification errors in estimates of harvest rates and run size.

| Early summer | Number of years | Midsummer | Number of years | Late summer | Number of years |
|---|---|---|---|---|---|
| Momich | 6 | Chilko | 33 | Lower Adams | 33 |
| Upper Adams | 1 | Horsefly | 17 | Lower Shuswap | 16 |
| Scotch | 11 | Mitchell | 8 | Middle Shuswap | 6 |
| Seymour | 33 | Stellako | 33 | Little | 3 |
| Upper Pitt | 29 | Middle | 22 | South Thompson | 2 |
| Gates | 22 | Tachie | 17 | Cultus | 30 |
| Fennell | 12 | Tesako | 15 | Harrison | 32 |
| Raft | 31 | Nithe | 2 | Birkenhead | 33 |
| South Chilko | 10 | | | Big Silver | 7 |
| Bowron | 21 | | | Portage | 19 |
| Early Nadina | 16 | | | Widgeon Slough | 20 |
| Late Nadina | 30 | | | | |

fishery targeting on the dominant Quesnel Lake run (a mid-timed stock) since 1981. Thus, misleading stock composition estimates may be obtained by excluding stocks a priori on the basis of spurious run timing assumptions.

*Simulation design*

We attempted to reconstruct potential stock identification errors in estimates of annual run size and harvest rates of Fraser River sockeye using simulations designed to mimic historical stock composition analyses. Our simulations considered only the potential for bias that arises because scale patterns can be similar among candidate stocks in a particular year. We used the run timing classification scheme used by our predecessors, although we expect that such a priori assumptions introduce an additional source of bias.

The magnitude and direction of bias in estimated stock proportions were assessed for simulated mixtures of known composition using maximum likelihood mixture analysis (Fournier et al. 1984). This technique has gained wide acceptance in stock identification problems using genetic and parasite data (Beacham et al. 1985a, 1985b, 1987; Bailey and Margolis 1987; Millar 1987; Wood et al. 1987, 1989; Mulligan et al. 1988). Simulations were restricted to the 1952–84 period to examine potential stock identification errors associated with Henry's (1961) method. The composition of simulated mixtures was based on (1) the reported (nominal) catches of each stock in each year (i.e., based on Henry's (1961) method), hereafter called the "nominal mixture" scenario, and (2) hypothetical catches computed from a constant harvest rate equal to the mean (1952–84) historical harvest rate, hereafter called the "constant harvest rate" scenario.

Reported catches for each stock used in the nominal mixture scenario may themselves be biased and, depending on the relative stock size and the degree of overlap in the distribution of scale characters among stocks, will either overestimate or underestimate the true catches. We recognize that the bias in estimates of catch due to stock misallocation may be exaggerated if larger stocks are overrepresented in the stock mixture and smaller stocks are underrepresented.

Artificial stock mixtures constructed using the constant harvest rate scenario were used to test the null hypothesis that the true harvest rates are constant. For this scenario, stock mixtures were constructed from catches estimated according to the following:

$$C_{ij} = h_i R_{ij}.$$

Here the catch $C_{ij}$ for stock $i$ in year $j$ is related to the mean stock-specific harvest rate $h_i$ and run size $R_{ij}$. Run size $R_{ij}$ is computed by dividing the escapement for stock $i$ in year $j$ by $1 - h_i$.

To evaluate the size of potential biases associated with historical analyses, we followed the conventional approach of classifying Fraser River sockeye stocks into discrete run timing groups, so that the number and identity of stocks forming the mixture were known a priori. Simulations were confined to stocks in the early summer, midsummer, and late summer run timing groups (Table 1). Excluded from the analysis is the early Stuart Lake run, the earliest-timed stock in the Fraser River watershed. We assumed that early Stuart Lake sockeye exist as a discrete run timing group with no overlap with other later timed stocks. The early summer run consists of a relatively large number of small stocks, of which Seymour River and Gates Creek sockeye exhibit pronounced 4-yr population cycles. The midsummer run consists of a few large populations including Quesnel Lake and late Stuart Lake stocks, two DSLL cyclic populations, as well as the relatively large Chilko River and Stellako River stocks. Late Shuswap Lake and Cultus Lake stocks are the principal stocks in the late-summer run that exhibits 4-yr cycles.

For each year and timing group, the total number of stocks in the reference and mixture samples varied depending on the number of discrete spawning populations sampled (Table 1). Furthermore, to reduce the effect of small sample sizes on stock identification errors, only stocks with sample sizes ≥30 fish were included in the simulations. Mixture sample size was fixed at 240 fish to simulate the sampling regime reported in Woodey (1987). Prior to analysis, principal components analysis was performed on the scale characters to eliminate correlations among scale variables, and the empirical distribution of scale characters was normalized to reduce errors resulting from discontinuous scale distributions, as described by Wood et al. (1989).

Mixtures used in the simulations were constructed from catches of age-4 sockeye from each stock. For most stocks,

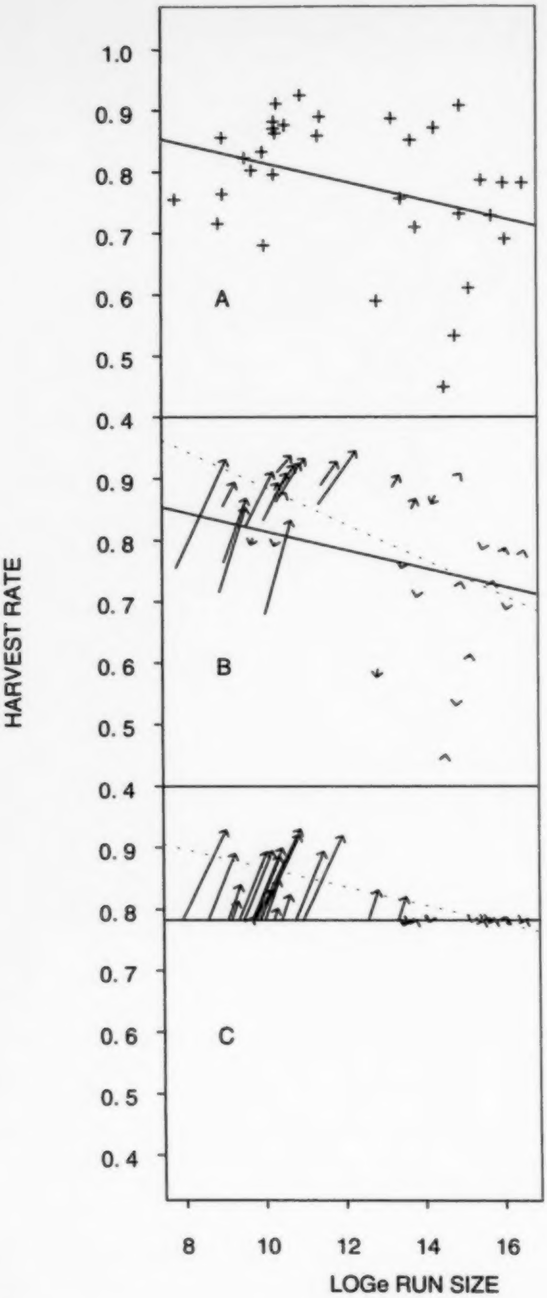

FIG. 5. Harvest rate – run size regressions for late Shuswap Lake sockeye (1952–84) showing relationships for (A) nominal data and (B) magnitude and direction of bias (arrows) from stock identification errors in estimates of harvest rate and run size for simulations under the nominal mixture scenario and (C) the constant harvest rate scenario. The solid lines in Fig. 5B and 5C are regression lines fitted to harvest rates and run sizes for known (simulated) mixtures. The broken lines in Fig. 5B and 5C are regression lines fitted to biased harvest rates and run sizes estimated by stock composition analysis of the simulated mixtures.

sample sizes of other age groups were too small to construct representative distributions of stock identification characters. For each year and run timing group, the mean of 50 bootstrapped estimates of stock proportions was used to represent the estimated stock proportions of each stock. Both the reference and mixture samples were randomly resampled to simulate sampling error. Stock proportions for all ages combined were then calculated from the following:

$$P_i = \left(\frac{c_4}{C}\right)\left(\frac{p_i}{q_i}\right),$$

where $P_i$ is the proportion of stock $i$, all ages combined, $p_i$ is the proportion of age-4 fish for stock $i$ estimated from the mixture model, $C$ is the total adult reported catch, $c_4$ is the age-4 reported catch, and $q_i$ is the proportion of age-4 spawners for stock $i$.

### *Simulation results*

Statistical evidence for the fishing hypothesis, based on nominal harvest rate – run size regressions, exists only for late Shuswap Lake sockeye. This relationship is shown in Fig. 5A for the 1952–84 period, for which estimates of catch were based primarily on Henry's (1961) method. The negative slope of this relationship is statistically significant ($p < 0.05$). The annual estimates of the harvest rate – run size data points are positively biased in 15 of the 17 off-cycle years, as depicted by the direction of the arrows. For the off-year cycles, the bias in harvest rates averaged 6% whereas the dominant and subdominant years' harvest rates are relatively unbiased and errors averaged less than 1% (Table 2). On average, biases of this magnitude are likely acceptable for in-season harvest management of Fraser River sockeye. However, the magnitude and the direction of bias, especially the positive bias in the off years, have serious implications for assessing evidence for depensatory fishing. The positive bias in the off years increases the negativity of the slope of the harvest rate – run size regression ($p < 0.05$) as depicted by the broken line in Fig. 5B. Note that in this scenario, simulations mimicked procedures used to derive the nominal catch data. Assuming that our attempt to mimic historical procedures is representative, the nominal data in Fig. 5 must be biased to approximately the same degree and in the same direction as our estimates. The *true* relationship between harvest rate and run size must be correspondingly less negative, and almost certainly, the slope of the relationship would not be statistically significant.

Similar results were obtained by analyzing simulated mixtures under the constant harvest rate scenario, our null hypothesis (Fig. 5C). For the two off-year cycle lines, the average bias in harvest rates was 9% whereas for the dominant and subdominant years the average bias was less than 1% (Table 2). The slope of the harvest rate – run size regression is significantly negative ($p < 0.01$), in fact more so than that calculated from the nominal data (Fig. 5A). Therefore, the relationship evident in the nominal data could be generated solely by stock identification errors. Consequently, the empirical evidence in support of the fishing hypothesis cannot be used as unequivocal evidence that depensatory fishing has occurred.

Simulation results for the remaining cyclic stocks indicate a wide range of potential effects of stock identification bias in estimates of harvest rate and run size (Fig. 6; Table 2). As discussed earlier, outliers in the nominal data for late Stuart

TABLE 2. Bias in reconstructed estimates of harvest rates by cycle line for cyclic Fraser River sockeye stocks. Bias is expressed as the mean deviation (as proportion) in estimated harvest rates from known (simulated) harvest rates (n = nominal mixture scenario and c = constant harvest rate scenario). $\beta$ is the slope of the linear regression of annual harvest rates on the natural logarithm of adult returns ($^*p < 0.05$; $^{**}p < 0.001$) for historical (nominal) data and simulated under the constant harvest rate scenario.

| Stock | Dominant | | Subdominant | | Low | | Low | | $\beta$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | *c* | *n* | *c* | *n* | *c* | *n* | *c* | Historical | Simulated |
| Late Shuswap | 0.0 | 0.0 | 0.0 | 0.0 | 0.07 | 0.08 | 0.05 | 0.10 | −0.015* | −0.015** |
| Quesnel | −0.03 | −0.05 | 0.14 | 0.14 | — | — | 0.12 | 0.20 | −0.008 | −0.046** |
| Late Stuart | 0.01 | 0.0 | 0.01 | 0.01 | 0.11 | 0.11 | 0.08 | 0.12 | −0.002 | −0.017 |
| Stellako | −0.03 | −0.03 | −0.03 | −0.03 | 0.02 | 0.03 | −0.04 | −0.04 | 0.029 | 0.031 |
| Seymour | −0.05 | 0.0 | 0.0 | 0.0 | 0.06 | −0.01 | −0.06 | 0.03 | 0.008 | 0.012 |
| Cultus | 0.10 | 0.05 | 0.01 | 0.08 | −0.04 | −0.03 | 0.03 | 0.0 | 0.040 | 0.026 |
| Chilko | −0.01 | −0.01 | −0.01 | −0.01 | 0.0 | 0.0 | 0.0 | 0.02 | −0.006 | −0.007 |
| Gates | −0.23 | 0.0 | 0.05 | 0.03 | 0.05 | 0.03 | 0.17 | 0.19 | 0.004 | −0.020 |
| Late Nadina | −0.10 | −0.05 | −0.13 | −0.04 | 0.01 | 0.02 | 0.05 | 0.10 | 0.004 | −0.026** |

Lake and Quesnel Lake sockeye reduce the degree of depensation in the fitted regression, thereby potentially masking evidence for depensatory fishing. Simulation results for Quesnel Lake sockeye resulted in highly significant negative harvest rate – run size regressions for both the nominal mixture and constant harvest scenarios ($p < 0.01$). The bias in harvest rate is particularly large for the subdominant cycle (1984) and the 1982 off cycle. This would cause harvest rates to be overestimated by up to 15% (Table 2). Conversely, average bias in estimates of harvest rates for the dominant-year line (1981) is −3 to −5%. For late Stuart Lake sockeye, the bias in estimates of harvest rates resulted in negative harvest rate – run size regressions, albeit statistically insignificant ($p > 0.05$).

Unfortunately, we were unable to assess stock identification errors for some key nondominant years for Quesnel Lake and late Stuart Lake stocks due to a lack of scale samples from the spawning grounds. For Quesnel Lake sockeye, no data are available for any year in the 1983 off-year cycle line, and only two years are available for the 1984 off-year line (1960 and 1964). Clearly, estimates of stock composition for years without data were based on procedures other than Henry's (1961) method. We do not know what effect the exclusion of these years had on our estimates of bias for the harvest rate – run size regressions. However, we would expect that adding more off years to the regression would increase the negativity of the slope of the regression owing to the larger proportional bias typical of nondominant years. It is more difficult to predict the effect of missing years in the harvest rate – run size regression for late Stuart Lake sockeye. Nevertheless, the magnitude and direction of bias in estimates of harvest rates and run size for Quesnel Lake and late Stuart Lake sockeye serve to negate inferences of depensatory fishing mortality based on nominal data.

Although the slopes of the relationships are not statistically significant ($p > 0.05$), stock identification errors led to a positive bias (i.e., apparent compensation) in the harvest rate – run size regressions for Cultus Lake, Seymour River, and Stellako River stocks (Fig. 6). For these stocks, the positive trend in the relationship is contrary to the expected direction of stock identification bias. Typically, stock proportions (and harvest rates) are expected to be underestimated for stocks that predominate in the mixtures as noted for the late Shuswap Lake stock and other studies using genetic and parasite stock identification characters (Beacham et al. 1985a, 1985b). For Cultus Lake sockeye, the positive bias in harvest rates for the dominant-year line (e.g., 1983) occurs in the same cycle line as the subdominant late Shuswap Lake stock which comigrates with Cultus Lake sockeye. The subdominant late Shuswap Lake stock is on average about eight times larger than the dominant Cultus Lake stock. Therefore, subdominant late Shuswap Lake sockeye will account for a much larger proportion of the stock mixture than the dominant line of Cultus Lake sockeye. Any misallocation of Shuswap Lake sockeye would potentially result in a positive bias in estimates of catch of the Cultus Lake stock. For Seymour River and Stellako River sockeye, the positive harvest rate – run size regressions are more related to the effect of outliers in the regressions than to a systematic bias in estimates of harvest rate or run size.

For the Gates Creek stock, the harvest rate – run size regression for the nominal mixture scenario is influenced by two particularly large outliers (Fig. 6). In this relationship the outliers result in a positive relationship between harvest rate and run size, albeit not statistically significant ($p > 0.05$). For the constant harvest rate scenario, the slope of the harvest rate – run size regression for Gates Creek is slightly negative but not significantly different from zero ($p > 0.05$). Apart from the effect of the outliers in the nominal mixture scenario, the overall direction of bias in both scenarios for Gate Creek sockeye, as depicted by the direction of the arrows, implies an overestimation of harvest rates at low run sizes (Fig. 6). For the late Nadina River stock, both the nominal mixture and constant harvest rate scenarios suggest that harvest rates are typically overestimated at low run sizes and underestimated in years of large runs. The slope of the harvest rate – run size regression under the constant harvest rate scenario was significantly negative ($p < 0.01$).

For the Chilko River stock, the slopes of the harvest rate – run size regressions for both the nominal mixture and constant harvest rate scenarios are statistically indistinguishable from zero ($p > 0.1$). Of all populations evaluated in this analysis, the bias in harvest rates was the lowest for the Chilko River stock with mean biases of less than ±2% among cycle lines (Table 2).

Unfortunately, the feasibility of correcting for bias in mixture problems of this nature to assess the true relationship

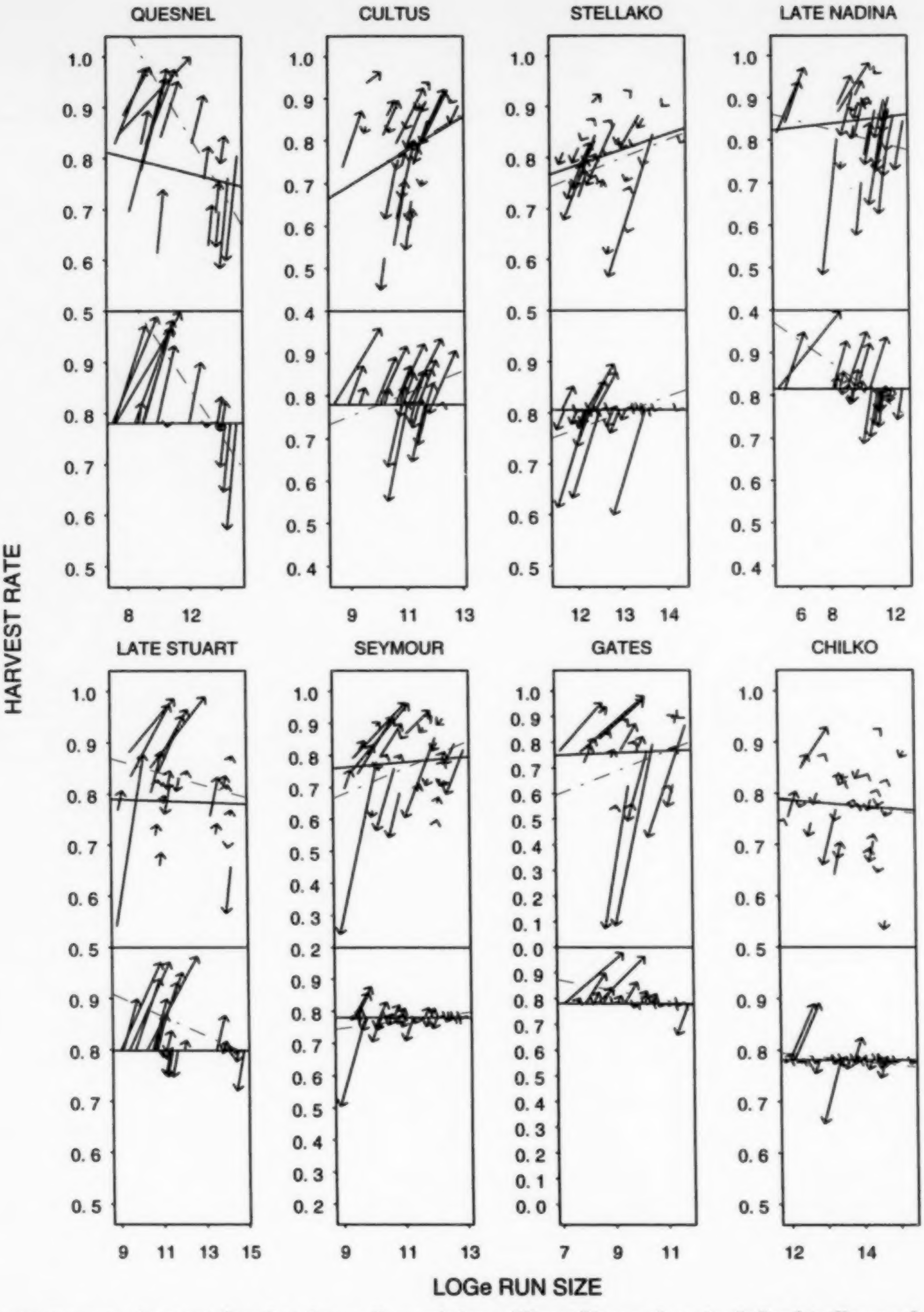

FIG. 6. Harvest rate – run size regressions for other cyclic populations of Fraser River sockeye (excluding late Shuswap Lake) under the nominal mixture scenario (upper panels) and constant harvest rate scenario (lower panels). Arrows and lines as described in Fig. 4.

between harvest and run size depends on the number of stocks in the mixtures. In some estimation procedures, bootstrap resampling can be used to correct for bias (Efron 1982). However, attempts to correct for bias using bootstrap resampling have failed when mixtures comprise more than two stocks (Millar 1987; Pella and Millar 1987). In this

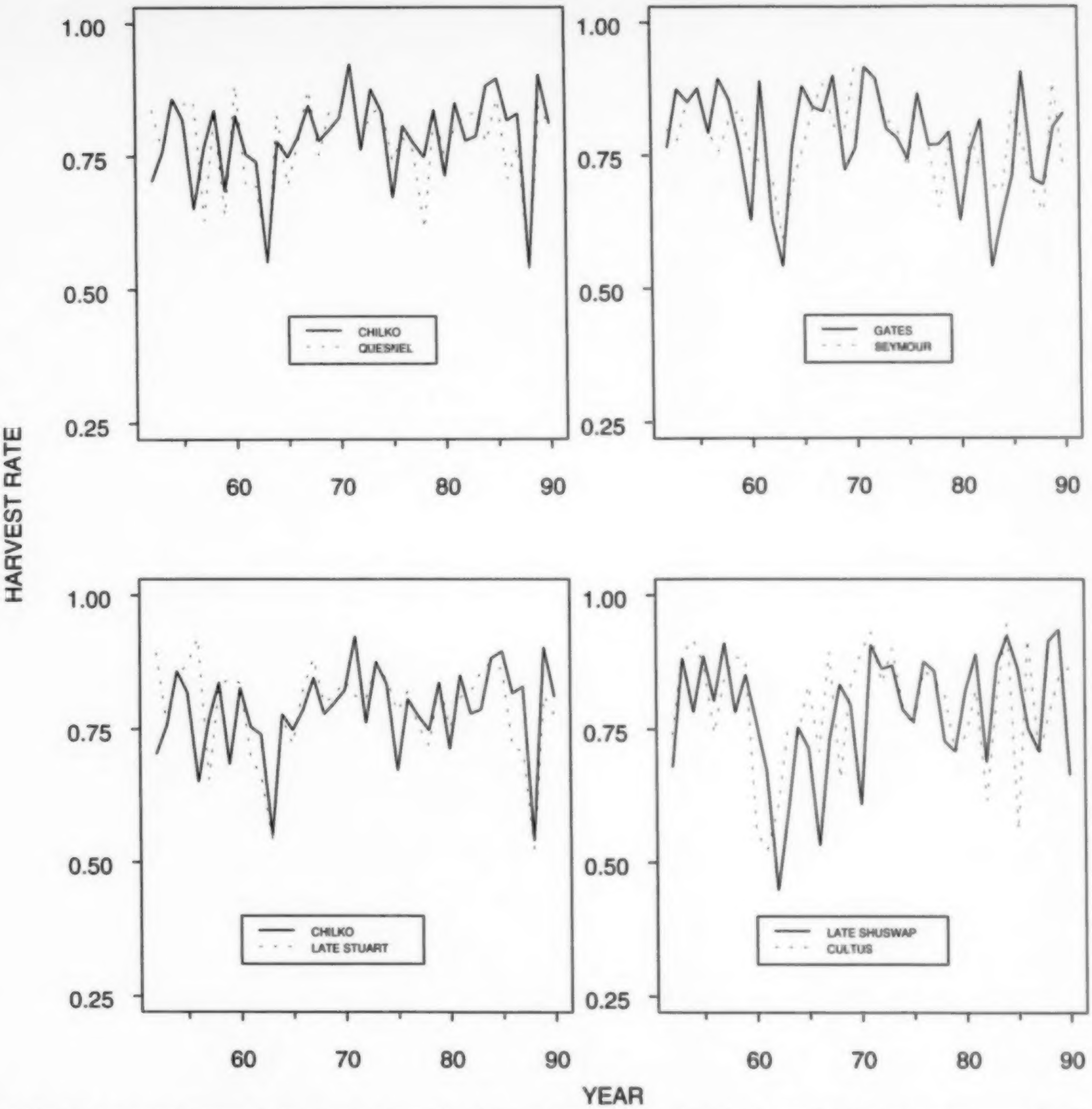

FIG. 7. Trends in nominal (reported) harvest rates in stocks of Fraser River sockeye that migrate together through the commercial fisheries.

particular mixture problem, there were 4–10 stocks in each mixture. Because of the multistock and multiyear complexity of this problem, we have not attempted to use bias-correction procedures.

### Harvest Rates and the Asynchrony of Cyclic Dominance

Since the 1940s, several upper Fraser River stocks have exhibited 4-yr cycles, but their dominant lines have occurred in different years. For the fishing hypothesis to explain this pattern, fisheries must exploit each stock at different rates in the same year. Several of these stocks migrate together through commercial fisheries (Killick 1955; Verhoeven and Davidoff 1962). It is difficult to understand how commercial fisheries could harvest comigrating stocks selectively enough to impose different and depensatory mortalities on each stock in the same year, as required to maintain the asynchronous cycles. For example, the dominant Chilko River line comigrates with the off-year late Stuart Lake and Quesnel Lake lines, and the off-year Chilko River line comigrates with the dominant-year Stuart Lake and Quesnel Lake lines. Indeed, cyclic populations within the early summer (Gates Creek and Seymour River), midsummer (Chilko River, late Stuart Lake, and Quesnel Lake), and late summer run timing groups (late Shuswap Lake and Cultus Lake) have parallel trends in harvest rates throughout much of the historical time series (Fig. 7).

We examined the association between nominal harvest rates sustained by several pairs of comigrating stocks including Chilko River versus Quesnel Lake, Chilko River versus late Stuart Lake, Gates Creek versus Seymour River, and late Shuswap Lake versus Cultus Lake (Fig. 8). In each comparison, the stocks migrate together in the same run

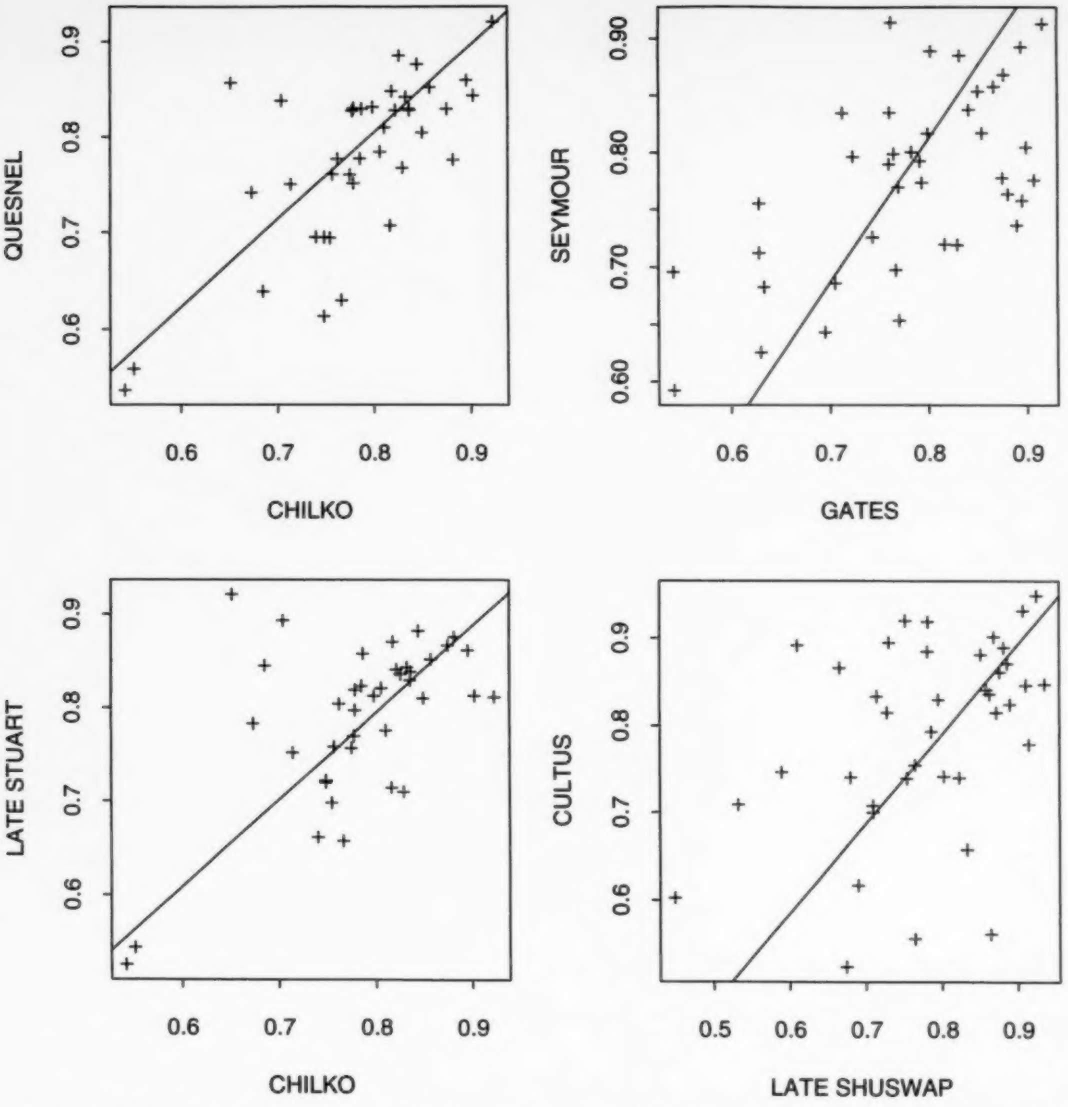

FIG. 8. Correlations between harvest rates in stocks of Fraser River sockeye that migrate together through the commercial fisheries. The line is the best fit to an errors-in-variables model assuming equal variances in harvest rates for the comigrating stocks.

timing group and are cyclic, but their dominant lines return in different years. The data were fit to an errors-in-variables model (Fuller 1987, p. 30–48) assuming equal variances in estimates of harvest rates for each stock. If the fishery harvests these comigrating but asynchronous stocks at different rates in the same year, as required to save the fishing hypothesis, the relationship between their harvest rates should be negatively correlated or at least have a slope indistinguishable from zero. However, for all comparisons, the slopes were significantly positive ($p < 0.05$). In two of the four comparisons (e.g., Chilko River versus late Stuart Lake and late Shuswap Lake versus Cultus Lake) the 95% confidence intervals of the slope included a slope of 1 (i.e., 1:1 correlation). We conclude therefore that the nominal harvest rate data for these stocks are inconsistent with the fishing hypothesis.

### Aboriginal and Early Commercial Fisheries

Considerable evidence has been compiled to suggest that the historic (1901) dominant cycle line, destroyed by rock dumping during railroad construction at Hell's Gate in 1913–14, had persisted since the time of the first Hudson Bay Company records (1820) and perhaps since the first reference to Fraser River sockeye (1793) (Ricker 1950, 1987; Ward and Larkin 1964; Roos 1991). Ward and Larkin (1964) compiled Hudson Bay Company records prior to 1873 from five different forts situated on major tributaries of the Fraser River. These records indicate that all the major

Fraser River sockeye runs exhibited 4-yr cycles, with the dominant cycle occurring in the same year. Later documents of the British Columbia Department of Fisheries also confirm that escapements to upper Fraser River populations cycled in synchrony between 1901 and 1913 (Ward and Larkin 1964). Although these records are qualitative, their concordance is too remarkable to dismiss. One should also recognize that salmon were a critically important food both for the aboriginal populations and for Hudson Bay Company traders, so that changes in salmon abundance would almost certainly be followed with interest.

If the historic dominant cycle did persist from the early 1800s, this persistence requires explanation. Commercial harvests were small prior to 1860, and it seems very doubtful that aboriginal fisheries were intensive enough to maintain cycles in sockeye abundance (Roos 1991). The fishing techniques available were primitive: traps or trawls extending a few feet below the surface and suspended between canoes were used in the lower Fraser River and estuary (Suttles 1951); large dip nets and harpoons were deployed from river banks in the Fraser canyon (Teit 1900); lattice-work weirs set against piles were effective in turbid streams of small to moderate size (e.g., Stuart and Nautley rivers (Kew 1992)). Kew (1992) emphasized that salmon were less accessible to aboriginal fisheries below as opposed to above the Fraser canyon. This is a significant point because it seems unlikely that the aboriginal trap, trawl, dipnet, and harpoon fisheries in the lower river or Fraser canyon could have been sufficiently intense to maintain cycles in sockeye abundance. Weir and dipnet fisheries in smaller tributaries to the upper Fraser River could have been more intense, but these exploited individual spawning runs. Again, it seems unlikely that these fisheries could have been conducted so as to maintain synchronous cycles in all major sockeye-producing tributaries. Some anthropologists (e.g., Hewes 1973; Romanoff 1985; Kew 1992) have suggested that salmon utilization by aboriginal people was also limited by their ability to process and preserve fish.

Following the construction of the first cannery on the Fraser River in 1866, the commercial gillnet fishery developed rapidly (Roos 1991). Reconstructed estimates of commercial salmon harvests and escapements from 1889 to 1913 indicate that sockeye abundance on the historic (1901) dominant cycle was consistently higher than in all other years (Gilhousen 1992). On average, within any 4-yr period prior to 1913, the dominant cycle was about six times larger than the subdominant (1902) cycle, nine times larger than the first off-year (1903) cycle, and 12 times larger than the second off-year (1904) cycle. Although these reconstructed run sizes are only approximate, it is interesting to note the similarity of this pattern to that for Shuswap Lake after 1926.

Roos (1991) and Gilhousen (1992) both pointed out that the early commercial gillnet fishery was intense and that harvest rates (excluding U.S. trap catches) on the nondominant cycles were higher (average around 70%) than on dominant cycles (average around 50%). Thus, it seems likely that cyclic trends in Fraser River sockeye production prior to 1913 were exaggerated and at least partially maintained by depensatory fishing. Owing to obstructions in the canyon at Hell's Gate, most sockeye returning in 1913 and 1914 failed to reach their spawning grounds (Roos 1991) and consequently, the 1901 dominant cycle was lost. Cyclic trends did not occur again in any population until 1926, when the current dominant cycle for Shuswap Lake sockeye first became evident (Larkin 1971).

## Alternative Mechanisms

Hypotheses to explain cyclic behaviour in Fraser River sockeye centre on identifying agents of depensatory mortality. Various ecological hypotheses have been proposed (Ricker 1950; Ward an Larkin 1964; Larkin 1971). Until recently the favoured ecological explanation for cyclic dominance was the Ward and Larkin (1964) lacustrine predation hypothesis. This hypothesis has not been refuted, but neither has it received much empirical support except that provided by Ward and Larkin (1964) concerning changes in condition factor and diet of rainbow trout (*Oncorhynchus mykiss*) from dominant to low year cycles in Shuswap Lake. Subsequent studies by Williams et al. (1989) indicated that although rainbow trout are, as individuals, the most voracious predator on sockeye fry, other more abundant species such as northern squawfish (*Ptychocheilus oregonensis*) consume more sockeye fry overall. Certainly, northern squawfish are capable of greatly reducing sockeye fry-to-smolt survival (Foerster and Ricker 1941). Extensive feeding studies on Shuswap Lake indicate that mortality from fish predators is compensatory in dominant and subdominant years, not depensatory as assumed in the predation hypothesis (Williams et al. 1989). However, it is still possible that predation may exert a higher mortality rate in the off years than in the dominant or subdominant years.

The asynchrony of cycles in individual spawning populations that rear in Shuswap Lake, for example Adams River, lower Shuswap River, and Seymour River versus Scotch Creek and Eagle River, suggests that any ecological mechanism, such as predation, must be localized near the spawning streams, or specific to particular runs (Levy and Wood 1992). Since 1988, the relative abundance of juvenile sockeye and kokanee has been estimated using electrophoretic techniques (C.C. Wood, J.M.B. Hume, and C.J. Foote, Pacific Biological Station, Nanaimo, BC V9R 5K6, unpublished data). These preliminary data also indicate that kokanee populations in Shuswap Lake do not fluctuate in synchrony with sockeye populations, as would be expected if lacustrine predation maintained cycles. Thus, the predation hypothesis cannot be considered a satisfactory alternative to the fishing hypothesis, unless there is reason to believe that depensatory mortality is inflicted by predators in the spawning stream or immediately after emergence while the various stocks are still distinct.

Other ecological factors are perhaps less attractive explanations for population cycles (see Levy and Wood 1992). Marine factors have largely been discounted because of the perceived inability of marine influences to generate asynchronous cycles among Fraser River sockeye populations (Ricker 1950). Depensatory mortality resulting from negative influences of dominant runs on subsequent food availability in nursery lakes seems unable to account for the DSLL cyclic pattern. If food availability is responsible for cyclic population behaviour, then the effect of dominant runs would be greatest in years following dominant runs. Moreover, there is no evidence that growth is reduced in years of low sockeye abundance. Rather, the greatest effect of population density is exerted in years of high sockeye abundance where growth rates are negatively correlated with juvenile sockeye densities (Goodlad et al.

1974; Williams et al. 1989). Disease or parasites could conceivably induce depensatory mortality in sockeye, but no mechanisms have been proposed to explain how.

The surprising collapse of the Frazer Lake sockeye run in Alaska, following a period of very rapid increase in abundance, was caused by ecological factors associated with overstocking the nursery lake, not overexploitation (Kyle et al. 1988). Kyle et al. (1988) suggested that oscillations in sockeye returns during a period of increasing escapements are evidence of population cycles in Frazer Lake sockeye, thus providing empirical evidence that interactions between year classes can result in catastrophic changes in survival. There is also some statistical evidence for interactions between year classes in the Adams River run (Collie and Walters 1987; Welch and Noakes 1990), although these may be especially difficult to detect while the low cycle year abundance is so small compared with the dominant year in that stock. Our results indicate that stock identification errors in time series of catches are likely to confound statistical evidence for interactions between year classes.

Despite the lack of experimental evidence, a number of ecological mechanisms could potentially exert depensatory mortality. These include predation or limiting foraging immediately after emergence, disease, for example IHN carried by fry, or parasites with intermediate hosts that cause lags between fry density and numerical response by the infecting parasite stage. Walters and Woodey (1992) concluded from their simulations that genetic factors could maintain population cycles or at least slow the recovery of off-cycle lines in Fraser River sockeye. The genetic model is conditional on high fishing mortality, a strongly heritable age at maturity, and age-dependent mortality with age-5 sockeye having a higher mortality than age-4 sockeye. As Walters and Staley (1987) pointed out, given the very high exploitation rate by the fishery, even rather subtle sources of depensatory mortality might prevent the off years from rebuilding.

## Conclusion

The fishing hypothesis is a simple and appealing explanation for cycles in abundance of Fraser River sockeye, but it is not an adequate explanation on its own. Our analysis indicates that fishing patterns since the 1950s have *not* imposed depensatory mortality on Fraser River sockeye. Nor do we think there is sufficient evidence to accept Walters and Staley's (1987) suggestion that aboriginal fisheries may have been sufficient to maintain the synchronous cycles of the 1800s. Only during the unregulated, early commercial fishery prior to the collapse of the upriver runs following the 1913–14 disaster at Hell's Gate is there any convincing evidence of depensatory fishing (Roos 1991; Gilhousen 1992). Of course, when it occurs, depensatory fishing mortality will reinforce existing cycles.

On the positive side, the fishing hypothesis has forced a careful review of harvest rates on Fraser River sockeye. Simulations such as those by Collie and Walters (1987), Walters and Staley (1987), Collie et al. (1990), and Welch and Noakes (1991) show emphatically that harvest rates on Fraser River sockeye have been excessive. Recent management actions have reduced target harvest rates to around 67–70% which is considered optimal for overall production. However, many of the low years are so depressed that experimental reductions in harvest rate to 50% for some populations have been considered to speed rebuilding and at the same time to assess the strength of ecological interactions between dominant and low year runs. Because off-year cycles are currently so small, rapid rebuilding is almost certainly desirable, even if ecological factors will eventually limit the magnitude of nondominant-year cycles. To our knowledge, all analyses to date suggest that rebuilding off-year runs as quickly as possible will increase overall production and economic benefits from Fraser River sockeye (e.g., Collie and Walters 1987; Walters and Staley 1987; Collie et al. 1990; Welch and Noakes 1991). In the meantime, we should not abandon research on the nature of ecological interactions between year classes that may have maintained current cyclic patterns for almost a century, and pre-Hell's Gate patterns since at least 1829, presumably at larger average run sizes.

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## Appendix

Simulations to assess the effects of harvest rates on the rebuilding and recovery of runs of Fraser River sockeye following a single catastrophic reduction were performed using a Ricker stock–recruitment function with log-normal error and nonoverlapping year classes of the form

$$(1)\quad R_t = S_{t-1}e^{a-bS_{t-1}+\sigma\epsilon}.$$

Here, run size $R_t$ in generation $t$ is related to spawning escapement $S_{t-1}$ in generation $t - 1$. The productivity parameter $a$ and the density-dependent parameter $b$ are those reported for Adams River sockeye in Cass (1989). The productivity for Adams River sockeye is considered to be typical of most other Fraser River sockeye stocks (Collie et al. 1990). The stochastic variation was fixed using a residual standard deviation $\sigma$ of 1.0, a level of noise within the range for Pacific salmon (Walters 1985; Collie and Walters 1987; Cass 1989; Welch and Noakes 1991), where $\epsilon$ is a standard normal deviate. Spawning escapement $S_t$ in generation $t$ is determined from the impact of harvest rate $h_t$ on subsequent run size where

$$(2)\quad S_t = R_t\,(1 - h_t).$$

In the simulations, the population was harvested for 12 generations at 75%, the harvest rate resulting in the maximum equilibrium run size, to allow populations to stabilize. A catastrophic reduction in abundance equal to the average difference between the dominant- and off-year cycles observed from 1952 to 1990 for Adams River sockeye (approximately 10 000 000 versus 10 000 sockeye) was introduced after the twelfth generation. During the ensuing 10 generations, the approximate length of reliable spawner-return estimates for most Fraser River stocks, populations were fished in separate simulations at sustained harvest rates of (i) 75%, (ii) 80%, near the 1952–80 mean harvest rate for cyclic populations, and (iii) 90%. Simulated populations fished at the same harvest rates but without a catastrophic reduction were used as a benchmark to assess the rate of recovery for cycle lines at the various harvest rates following the reduction (Fig. 1). The run size for each generation was computed from the mean of 500 simulations (Monte Carlo trials). At sustained harvest rates of 75 and 80% following the reduction, the abundance rebuilds to near the maximum run size in about six generations. At sustained harvest rates of 90%, cycle lines decimated by catastrophe will be held near the level of off-year cycles. However, high sustained harvest rates (90%) not only prevent off-year cycle lines from rebuilding, they also reduce the run size of dominant-year cycles to low equilibrium stock sizes, thereby eliminating cycles. These simulations show how populations respond on average to catastrophe under different harvest scenarios.

Under the (deterministic) Ricker recruitment function, sustained harvest rates above 80% will drive the Adams River population to an equilibrium below the average subdominant level (Fig. 2). Note that even a small absolute increase in catch at small run sizes significantly increases the harvest rate, thereby reducing the equilibrium run size.

To compare empirical frequency distributions of run size for cyclic populations with frequency distributions of run size expected without depensatory fishing or other mechanisms causing cycles, a second set of simulations was performed using Eqs. 1 and 2. As before, populations were allowed to stabilize near the maximum equilibrium run size by fishing at a sustained harvest rate of 75% for 12 generations. Separate simulations were performed using two levels of environmental noise ($\sigma = 0.5$ and $\sigma = 1.0$). Following a catastrophic reduction in run size (e.g., three orders of magnitude), populations were fished at harvest rates of 80 and 90% for 10 generations. Histograms of the mean run size in the last three generations are plotted in Fig. 3 to compare simulated run size distributions with run size distributions actually observed in cyclic populations. To facilitate comparison, the simulated and observed data were rescaled to express run size relative to the theoretical run size producing maximum sustainable yield ($R_{opt}$) in each stock. That is, run size in each stock was divided by its corresponding $R_{opt}$ computed from a pooled Ricker "$a$" parameter and stock-specific density-dependent parameters (Collie et al. 1990). Density-dependent recruitment parameters for the simulated populations are for Shuswap Lake sockeye. Histograms for simulated data are from 500 trials. The observed data are for Shuswap Lake, Quesnel Lake, and Stuart–Takla–Trembleur Lake populations that have cycled persistently since 1952. The data are scaled logarithmically such that each histogram bin represents an order of magnitude.

The purpose of these simulations was to demonstrate that the distributions of run size for cyclic populations (Fig. 3E or 3F) do not resemble simulated run sizes generated without a depensatory mechanism controlling population abundance (Fig. 3A–3D). For the real population data, off-year cycle lines are represented by a mode in the histogram nearly six orders of magnitude below that of the dominant cycle lines. Subdominant cycle lines overlap the distributions of both off and dominant lines but are represented by a mode two orders of magnitude below that for the dominant lines. In contrast, frequency distributions for the simulated populations are unimodal and do not resemble the empirical data. At sustained harvest rates of 80%, runs rebuild to optimal levels (mode at $-1$ to $+1$). For $\sigma$ in the ranges observed for Pacific salmon, there is little chance that sustained harvest rates at the historical 1952–90 average (80%) will produce runs in the ranges observed in cyclic populations (e.g., both low and dominant runs). At harvest rates of 90%, runs are held below the optimal level (mode at $-2$ to 0). At sustained harvest rates of 90% and $\sigma = 1.0$, the range of the simulated data spans that for the observed data but there is no evidence of a cyclic pattern. Frequency distributions of run size for simulated populations were significantly different from the observed data ($G$-test (Sokal and Rohlf 1981); $p < 0.05$) under all combinations of harvest rate and noise level.

# Sedimented Diatoms in Northern New England Lakes and Their Use as pH and Alkalinity Indicators

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Gradient analyses of 29 chemical and physical (C–P) variables and diatom remains in surface sediments of 63 New England lakes (pH 4.4–7.9) indicate a primary C–P gradient of pH, alkalinity, Ca, Mg, conductance, and Al; diatom distributions are most strongly related to that gradient (especially to pH and alkalinity) and also reflect secondary gradients (oceanic–inland, lake morphology, and regional chemistry). The primary relationship supports the calibration of regression models for paleolimnological inference of pH and alkalinity based on diatoms. To optimize inference models for the region's most acidic lakes, a second set of calibrations was run after culling the seven least acidic lakes. Diatom distributions on the restricted pH and alkalinity gradients are of two types: roughly uniclinal and variously unimodal. Models assuming each type were calibrated: CLUSTER (linear), DCA (unimodal), and CCA (unimodal). Log-transformation of alkalinity improved the 63-lake DCA and CLUSTER regressions, but worsened or left the others unchanged. Postulated causes of incongruous diatom assemblages and outlier pH and alkalinity inferences are sediment mixing, focusing time lag, growth of epipelic diatoms at the core site, and atypical lake morphology. Careful selection of calibration lakes is at least as important as the choice of regression models.

Des analyses de gradients de 29 variables chimiques et physiques et les vestiges de diatomées dans les sédiments de surface de 63 lacs de Nouvelle-Angleterre (pH 4,4 à 7,9) indiquent un gradient primaire de pH, d'alcalinité, de Ca, de Mg, de conductivité et de Al; la distribution des diatomées est plus étroitement liée à ce gradient (notamment au pH et à l'alcalinité) et reflète également des gradients secondaires (océanique–intérieur, morphologie des lacs et propriétés chimiques régionales). La relation primaire justifie l'étalonnage de modèles de régression permettant des déductions paléolimnologiques du pH et de l'alcalinité à partir des diatomées. Afin d'optimaliser les modèles de déduction pour les lacs les plus acides de la région, on a effectué une deuxième série d'étalonnage après avoir éliminé les sept lacs les moins acides. La distribution des diatomées dans les gradients limités de pH et d'alcalinité peut être classée en deux types : à peu près monoclinique et diversement unimodale. Des modèles ont été étalonnés pour chaque type : CLUSTER (linéaire), DCA (unimodal) et CCA (unimodal). L'expression de l'alcalinité sous forme de logarithme a amélioré la régression avec les modèles DCA et CLUSTER pour les 63 lacs, mais l'a empiré ou n'a aucun résultat pour les autres variables. Les causes probables de l'assemblage incompatible de diatomées et les mauvaises déductions du pH et de l'alcalinité sont le mélange des sédiments, le retard de mise au point, la croissance de diatomées épipélagiques au site de carottage et la morphologie atypique des lacs. Il est au moins aussi important de bien choisir les lacs pour l'étalonnage que de choisir les modèles de régression.

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Human and nonhuman effects on lake water chemistry and biology prior to the most recent few decades are poorly understood due to scarcity or absence of data and the suspect nature of some early analytical techniques. This situation has stimulated the development of paleolimnological methods based mostly on diatom and scaled chrysophyte remains, especially for the reconstruction of lake acidity, trophic state, and salinity, all of which can be related to other limnological variables (e.g., fishes) and exogenous forcing factors (e.g., acid deposition, climate change, and catchment processes) (Battarbee 1984; Davis 1987, 1989; Agbeti and Dickman 1989; Charles et al. 1989; Fritz 1990; Kingston et al. 1992; Renberg et al. 1993b; Rhodes and Davis 1995). Ongoing efforts to refine these methods and to include additional chemical variables, bioindicators, and geographic regions (Charles and Smol 1988; Birks et al. 1990; Kingston et al. 1992; Cumming and Smol 1993; Dixit et al. 1990, 1993) are highly relevant to current limnological problems, including the need for long-term and paleolimnological studies to separate human- from nonhuman-caused variation in lakes (Digerfeldt and Håkansson 1993; Naiman and Magnuson 1993; Renberg et al. 1993a; Davis et al. 1994).

To quantitatively infer past limnological conditions from sedimented bioindicators, inferential models with transfer coefficients are needed. The models, in the form of regression equations, must be calibrated, that is, coefficients must be quantified. For lake water chemistry, calibration is based on data sets consisting of bioindicator counts from surface sediments and contemporary water chemistry from many lakes in a region. Such calibration data sets have been developed for several regions of Europe and North America. Recent sets include those of Fritz (1990), Kingston et al. (1990), Sweets et al. (1990), Dixit et al. (1991, 1993), and Stevenson et al. (1991).

However, for northern New England (Maine, New Hampshire, and Vermont), U.S.A. (Fig. 1), a region with some of the earliest diatom-based quantitative paleolimnological reconstructions in North America (e.g., Davis et al. 1983), only partial analyses of abbreviated calibration sets have been published (Norton et al. 1981; Davis and Anderson 1985). This paper presents the full set that has been applied in recent paleolimnological studies in the region (Davis et al. 1990, 1994) plus an analysis of the potential influences of diverse environmental gradients on the distributions of the bioindicator diatoms (Results, Part 1). The set and analysis differ from others in North America in their coverage of steep altitudinal and maritime–inland gradients. The main goal of this analysis is calibration of regression models for inference of water pH and alkalinity (alk) from diatom remains in sediment cores from the region's most acidic lakes (Results, Part 2). In addition, this paper provides a limnological characterization of an important class of lakes in the region.

Inferential models that regress diatom data differ from each other in their assumptions regarding taxa distributions and in their mode of "packaging" the data. Linear models have been used to regress individual taxa (e.g., Davis and Anderson 1985), Hustedt pH groups of taxa or indices based on these groups (e.g., Davis and Berge 1980; DelPrete and Schofield 1981; Renberg and Hellberg 1982), clusters of taxa (Charles 1985; Davis et al. 1985), and principal components of taxa (Davis and Anderson 1985). Shortcomings of these and other linear models and the theoretical superiority of nonlinear models using weighted averages (WA) of taxon frequencies on environmental gradients were discussed by ter Braak and van Dam (1989) and Birks et al. (1990). In this paper, we calibrate one linear regression model and two WA models for inference of pH and alk. Elsewhere, we have discussed the autecology of the diatom taxa that are important in the calibrations and inferences (Anderson et al. 1993).

## Materials and Methods

### Study Sites and Rationale for Their Selection

We describe the diatom remains in surface sediments and the chemical and physical characteristics of 63 lakes in northern New England. The lakes were selected to include the ranges of limnological conditions that reasonably could be hypothesized for the past 300 yr in the 12 lakes where paleolimnological reconstructions were to be carried out in the region (Fig. 1; Table 1). These 12 lakes are small (2–23 ha, med. 10), relative deep ($z_r$ 1.8–9.6%, med. 3.5), acidic (pH 4.6–6.1, med. 5.1; alk −23 to 27 $\mu eq \cdot L^{-1}$, med. −3), oligotrophic (chlorophyll *a* 0.2–3.2 $\mu g \cdot L^{-1}$, med. 0.9), and have low to moderate water color (Pt–Co units 3–33; dissolved organic carbon (DOC) 0.2–7.3 $mg \cdot L^{-1}$). They have catchments with granitic bedrock and coarse-grained shallow soils and range in altitude from 67 to 1038 m a.s.l. Given these characteristics, we consider it unlikely that they would have had pH > 6.5 and alk > 100 $\mu eq \cdot L^{-1}$ in the past 300 yr. This assessment has been confirmed by the strongly acidophilic character of the lakes' down-core diatom assemblages (Norton et al. 1985; Davis et al. 1990, 1994).

As our main paleolimnological goal was inference of acidity, we selected calibration lakes to evenly cover the range of pH that might have occurred in the reconstruction lakes. To be sure that the range was fully covered, we included some of the more acidic lakes (not peatland lakes) as well as more alkaline lakes in the region. The 63 lakes range in altitude from 52 to 1123 m a.s.l. (med. 436). They have small surface areas (med. 6.0 ha), but are relatively deep (med. $z_r$ = 3.0%). They are oligotrophic to mildly mesotrophic: med. chlorophyll *a* 2.2 $\mu g \cdot L^{-1}$; med. total P 9.8 $\mu g \cdot L^{-1}$. These are low-salinity lakes (conductance 12–215 $\mu mho \cdot cm^{-1}$, med. 23) with low to moderate water color (Pt–Co units 3–103, med. 20; DOC 0.2–10.0 $mg \cdot L^{-1}$, med. 3.2), negative to low-positive alk (−40 to 1758 $\mu eq \cdot L^{-1}$, med. 27), and pH 4.4–7.9 (med. 6.0) (Tables 1 and 2). (In this paper, all pH values are non-air-equilibrated field determinations. We use the term alkalinity (alk) as a synonym for acid-neutralizing capacity (ANC), that is, total microequivalents of acid-neutralizing solutes per litre.)

The 63-lake set is not typical of the total population of lakes of northern New England as described by the USEPA Eastern Lake Survey (ELS) (Linthurst et al. 1986; Brakke et al. 1988); its pH distribution (Fig. 2) does not reflect the strong ~7 mode that characterizes the total population. The ELS suggests that only 1.7% of the lakes in northern New England have pH < 5 whereas 19% of our 63 lakes have pH < 5.

### Water Sampling and Chemistry

Most of the lakes were sampled three times in 1983 and four times in 1984, during the ice-free seasons, and in accord with protocols of the Paleoecological Investigation of Recent Lake Acidification (PIRLA) (Charles and Whitehead 1986;

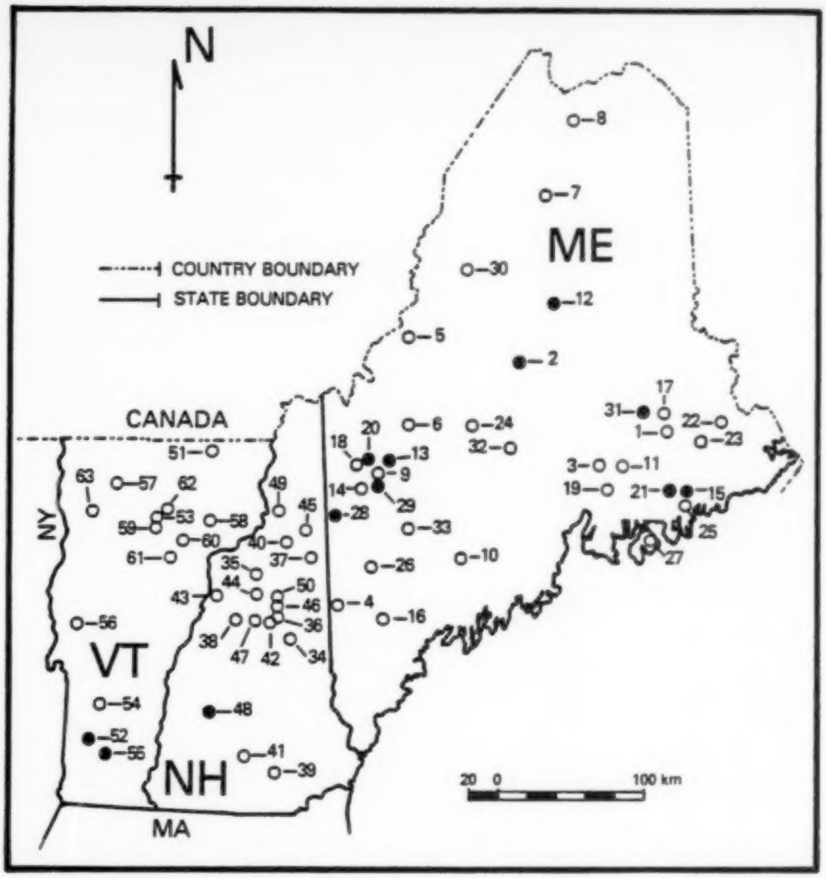

FIG. 1. Northern New England, with locations of 63 study lakes. The twelve solid circles indicate lakes from which sediment cores have been studied to infer histories of lake acidity ("reconstruction lakes" of Davis et al. (1983, 1990, 1994) and Norton et al. (1985)). Names and characteristics of numbered lakes are given in Table 1. MA, Massachusetts; ME, Maine; NH, New Hampshire; NY, New York; VT, Vermont.

Norton et al. 1986). Fourteen of the lakes in New Hampshire and Vermont were sampled only in 1984. For some lakes, occasional non-PIRLA data from 1978 to 1980 (Norton et al. 1981) and from 1980 to 1984 (Buso et al. 1984; Haines et al. 1984; Kahl et al. 1985) were added. The non-PIRLA data were collected in accord with USEPA protocols and, like the PIRLA data, were subjected to rigorous quality control and assurance.

Five tube samples of the mixed stratum of the water column over the deepest part of a lake and ~10 m apart were collected and pooled. Some aliquots of the pooled sample were filtered (one filter retained for chlorophyll *a* analysis), as appropriate for specific analyses. In the field, one unfiltered aliquot was used to determine pH using an Orion Ross combination electrode and a Corning 4T-mV-pH meter. Another unfiltered aliquot was used for extraction of exchangeable (exch) Al by the method of Driscoll (1984). Samples were kept refrigerated or iced during transport to the laboratory.

In the laboratory, alk was determined by Gran titration using an Orion Ross combination electrode attached to an Orion 701A-mV-pH meter. Cations were measured on a Perkin-Elmer 703 atomic absorption spectrophotometer, with air–acetylene flame (K, Na) or $N_2O$–acetylene flame (Ca, Mg), or graphite furnace (HGA 2200) for Al. Ammonium and anions were determined by ion chromatography (Dionex 2010i). Other methods were inductively coupled plasma emission spectroscopy for Si, infrared spectrophotometry for DOC, persulfate digestion, molybdate reaction, and spectrophotometry for total P, and acetone extraction and spectrophotometry using a trichromatic equation for chlorophyll *a*. Details on analytical procedures and quality assurance are given, respectively, in Norton et al. (1986) and Cook (1986).

### Collection of Surface Sediment, and Diatom Analysis

Surface sediment samples consisted of the top 0.5 cm of deposit from approximately the deepest location in each lake. From 41 of the lakes, sediment was collected with a 3- or 4-cm-diameter modified Hongve (1972) corer. At each of these lakes, surface sediments from two to five cores, 3–6 m apart, were combined. The remaining 22 lakes were cored with a modified Davis and Doyle (1969) piston corer (either 6.3 or 10.0 cm in diameter). In addition to providing

TABLE 1. Physical and chemical characteristics of the 63 lakes (lakes 1–33 in Maine, 34–50 in New Hampshire, and 51–63 in Vermont

| | pH | Alk (μeq·L$^{-1}$) | Cond (μmho·cm$^{-1}$) | Na (μeq·L$^{-1}$) | K (μeq·L$^{-1}$) | Mg (μeq·L$^{-1}$) | Ca (μeq·L$^{-1}$) | F (μeq·L$^{-1}$) | Cl (μeq·L$^{-1}$) | Mar-$SO_4$ (μeq·L$^{-1}$) | Non-mar-$SO_4$ (μeq·L$^{-1}$) | $NO_3$ (μeq·L$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Crystal P. | 4.96 | −10.0 | 14.6 | 37.8 | 3.5 | 15.0 | 24.7 | 0.7 | 27.1 | 2.7 | 53.1 | 0.7 |
| 2. East Chairback P.* | 5.12 | −9.1 | 12.2 | 25.1 | 3.7 | 26.2 | 33.9 | 0.7 | 24.4 | 2.4 | 72.2 | 2.0 |
| 3. Fitts P. | 6.24 | 36.7 | 24.5 | 70.1 | 7.4 | 28.6 | 83.2 | 13.7 | 41.9 | 4.2 | 85.6 | 0.4 |
| 4. Granger P. | 6.42 | 53.9 | 23.0 | 68.3 | 6.3 | 31.3 | 77.7 | 3.2 | 37.5 | 3.8 | 64.5 | 0.8 |
| 5. Heald P. | 6.70 | 141.2 | 29.2 | 28.1 | 6.0 | 64.6 | 163.0 | 1.3 | 7.8 | 0.8 | 79.5 | 1.8 |
| 6. (The) Horns P. | 6.00 | 35.2 | 19.0 | 22.1 | 7.5 | 32.6 | 77.3 | 1.1 | 14.5 | 1.4 | 73.1 | 1.7 |
| 7. Horseshoe P. | 6.82 | 217.8 | 37.5 | 25.3 | 7.3 | 59.1 | 236.5 | 0.9 | 10.8 | 1.1 | 77.7 | 2.0 |
| 8. Hunnewell L. | 7.41 | 385.6 | 50.6 | 45.0 | 4.7 | 70.2 | 359.3 | 1.3 | 7.8 | 0.8 | 58.3 | 1.0 |
| 9. Jackson P. | 4.75 | −15.2 | 20.0 | 30.9 | 4.0 | 17.0 | 40.2 | 1.3 | 9.2 | 0.9 | 73.1 | 1.7 |
| 10. Jimmie P. | 6.54 | 101.1 | 34.7 | 80.0 | 11.7 | 42.8 | 163.4 | 2.4 | 48.4 | 4.8 | 89.6 | 1.9 |
| 11. Jellison Hill P. | 5.91 | 26.1 | 24.1 | 75.4 | 5.0 | 32.8 | 75.6 | 5.6 | 50.9 | 5.1 | 79.3 | 2.3 |
| 12. Klondike P.* | 6.10 | 20.5 | 12.3 | 33.3 | 5.1 | 4.3 | 51.2 | 2.8 | 6.5 | 0.7 | 43.9 | 6.8 |
| 13. Ledge P.* | 5.25 | 5.2 | 17.8 | 17.6 | 4.2 | 19.7 | 81.3 | 1.2 | 13.2 | 1.3 | 74.3 | 1.8 |
| 14. Little Ellis P. | 6.48 | 67.1 | 24.1 | 52.5 | 10.9 | 46.5 | 95.4 | 1.5 | 15.2 | 1.5 | 99.0 | 0.8 |
| 15. Little Long P.* | 5.65 | 3.7 | 24.3 | 93.4 | 6.0 | 28.4 | 43.9 | 1.4 | 79.7 | 8.0 | 70.6 | 1.1 |
| 16. Little Sebago L. | 6.82 | 138.7 | 36.4 | 92.7 | 9.6 | 44.5 | 168.1 | 1.8 | 68.9 | 6.9 | 70.4 | 0.7 |
| 17. Loon P. | 5.95 | 25.9 | 14.8 | 33.4 | 9.6 | 14.1 | 53.4 | 0.4 | 25.7 | 2.6 | 34.8 | 0.8 |
| 18. Long P. (Beaver Mt.) | 6.21 | 31.4 | 20.4 | 41.8 | 8.5 | 31.8 | 81.5 | 1.7 | 19.0 | 1.9 | 89.2 | 1.7 |
| 19. Moulton P. | 6.88 | 148.7 | 36.4 | 88.8 | 11.5 | 52.8 | 161.6 | 6.1 | 59.0 | 5.9 | 75.5 | 3.4 |
| 20. Mountain P.* | 5.98 | 26.7 | 17.8 | 29.1 | 6.5 | 32.4 | 72.9 | 2.3 | 12.8 | 1.3 | 83.5 | 2.8 |
| 21. Mud P.* | 4.57 | −23.0 | 30.9 | 91.0 | 4.9 | 24.7 | 28.4 | 0.9 | 76.6 | 7.7 | 89.6 | 0.7 |
| 22. North Beaverdam L. | 6.32 | 44.3 | 18.9 | 61.1 | 7.2 | 26.1 | 58.3 | 0.9 | 41.1 | 4.1 | 43.3 | 0.9 |
| 23. Peep L. | 5.87 | 12.7 | 15.1 | 63.0 | 6.8 | 16.8 | 23.2 | 0.7 | 42.3 | 4.2 | 41.9 | 0.7 |
| 24. Piper P. | 6.53 | 72.8 | 22.0 | 42.7 | 9.0 | 41.1 | 92.4 | 0.4 | 22.6 | 2.3 | 59.2 | 0.9 |
| 25. Salmon P. | 6.53 | 48.4 | 21.0 | 83.6 | 6.8 | 30.4 | 62.8 | 5.0 | 70.9 | 7.1 | 46.1 | 0.1 |
| 26. Sand P. | 6.56 | 85.9 | 25.6 | 62.6 | 7.9 | 33.9 | 105.6 | 1.2 | 33.6 | 3.4 | 61.3 | 2.3 |
| 27. Sargent Mountain P. | 4.58 | −28.0 | 30.8 | 135.2 | 6.7 | 31.2 | 30.6 | 0.3 | 138.3 | 13.8 | 79.2 | 1.0 |
| 28. Speck P.* | 5.00 | −3.4 | 17.2 | 18.7 | 5.7 | 19.3 | 46.5 | 0.8 | 7.0 | 0.7 | 74.7 | 1.1 |
| 29. Tumbledown P.* | 5.20 | −1.7 | 14.2 | 18.0 | 2.6 | 22.5 | 43.1 | 0.8 | 6.2 | 0.6 | 71.8 | 2.2 |
| 30. Turner P. | 6.52 | 141.5 | 31.8 | 30.4 | 6.6 | 81.4 | 174.6 | 1.8 | 6.8 | 0.7 | 83.5 | 4.2 |
| 31. Unnamed P.* | 4.73 | −16.8 | 15.3 | 31.9 | 4.1 | 10.4 | 21.1 | 0.6 | 21.5 | 2.1 | 54.2 | 0.3 |
| 32. West Garland P. | 7.48 | 1137.0 | 129.9 | 75.7 | 11.8 | 218.7 | 1028.0 | 1.8 | 49.7 | 5.0 | 82.2 | 2.4 |
| 33. Worthley P. | 6.44 | 47.4 | 26.8 | 65.8 | 9.3 | 34.9 | 100.2 | 1.0 | 36.9 | 3.7 | 96.2 | 0.9 |
| 34. Bean P. | 5.63 | 16.5 | 14.5 | 40.9 | 12.6 | 16.2 | 58.0 | 9.8 | 12.5 | 1.2 | 66.3 | 4.0 |
| 35. Black P. | 6.14 | 57.0 | 18.5 | 58.7 | 14.8 | 27.2 | 98.9 | 25.0 | 11.0 | 1.1 | 74.9 | 5.9 |
| 36. Black Mountain P. | 5.44 | 10.1 | 19.7 | 28.7 | 8.7 | 22.5 | 65.1 | — | 18.8 | 1.9 | 111.6 | 1.5 |
| 37. Carter P. | 6.10 | 39.9 | 23.3 | 22.7 | 8.9 | 60.9 | 82.2 | 2.4 | 11.0 | 1.1 | 108.1 | 1.4 |
| 38. Cone P. | 4.57 | −26.1 | 23.9 | 22.1 | 1.9 | 14.0 | 36.8 | 2.6 | 11.7 | 1.2 | 130.1 | 1.1 |
| 39. Darrah P. | 4.51 | −39.7 | 24.9 | 40.2 | 9.3 | 18.8 | 29.7 | 0.4 | 39.9 | 4.0 | 83.3 | 1.0 |
| 40. Ethan P. | 5.25 | 19.3 | 18.5 | 50.6 | 7.3 | 20.8 | 94.9 | 46.3 | 16.0 | 1.6 | 87.4 | 4.0 |
| 41. Horton P. | 5.40 | 13.4 | 47.2 | 229.2 | 9.6 | 37.2 | 78.4 | 2.0 | 44.1 | 4.4 | 70.9 | 0.6 |
| 42. Kiah P. | 5.91 | 38.3 | 17.0 | 34.5 | 5.9 | 22.4 | 103.4 | 4.0 | 14.3 | 1.4 | 75.9 | 1.9 |
| 43. L. Constance | 4.79 | −10.5 | 19.0 | 27.6 | 5.4 | 18.1 | 68.0 | 8.0 | 15.0 | 1.5 | 123.3 | 2.2 |
| 44. Little East P. | 5.13 | −9.0 | 19.0 | 42.7 | 16.0 | 11.1 | 72.5 | 18.7 | 12.2 | 1.2 | 115.6 | 14.0 |
| 45. Lost P. | 5.84 | 25.8 | 15.0 | 19.8 | 6.6 | 47.3 | 71.8 | 5.0 | 12.5 | 1.2 | 84.8 | 4.8 |
| 46. Lower Greeley P. | 6.14 | 57.5 | 26.0 | 56.3 | 19.0 | 13.8 | 151.9 | 75.3 | 11.0 | 1.1 | 107.4 | 10.0 |
| 47. Middle Hall P. | 5.99 | 25.9 | 22.4 | 33.2 | 5.8 | 27.5 | 94.9 | 3.3 | 13.3 | 1.3 | 105.0 | 1.0 |
| 48. L. Solitude* | 4.83 | −15.3 | 19.5 | 26.6 | 5.5 | 18.2 | 44.5 | 1.7 | 14.4 | 1.4 | 89.0 | 0.9 |
| 49. Unknown P. | 4.45 | −39.3 | 14.5 | 9.1 | 2.6 | 5.1 | 12.8 | 1.0 | 5.0 | 0.5 | 51.2 | 1.4 |
| 50. Upper Greeley P. | 6.12 | 38.5 | 25.0 | 46.5 | 22.8 | 12.2 | 159.2 | 84.3 | 14.0 | 1.4 | 112.9 | 17.0 |
| 51. Beaver P. | 6.38 | 50.6 | 25.9 | 24.6 | 5.3 | 30.2 | 145.2 | 4.6 | 10.4 | 1.0 | 100.1 | 2.4 |
| 52. Branch P.* | 4.72 | −17.1 | 19.4 | 21.6 | 8.7 | 22.2 | 39.7 | 0.9 | 6.8 | 0.7 | 84.1 | 2.6 |
| 53. Goodall P. | 7.71 | 1368.0 | 126.5 | 37.7 | 6.3 | 137.1 | 893.2 | 5.3 | 20.3 | 2.0 | 95.0 | 5.3 |
| 54. Griffith P. | 5.74 | 23.0 | 19.1 | 18.4 | 8.6 | 31.8 | 89.3 | 1.2 | 9.0 | 0.9 | 82.6 | 1.2 |
| 55. Haystack P.* | 4.83 | −18.3 | 19.5 | 17.1 | 3.9 | 20.0 | 41.0 | 0.5 | 11.5 | 1.1 | 72.4 | 5.7 |

(Fig. 1)). The 12 lakes for which paleolimnological reconstructions were carried our are indicated by asterisks. L = Lake; P = Pond.

| $NH_4$ (μeq·L$^{-1}$) | Total Al (μg·L$^{-1}$) | Exch Al (μg·L$^{-1}$) | Total P (μg·L$^{-1}$) | $SiO_2$ (mg·L$^{-1}$) | Chl *a* (μg·L$^{-1}$) | DOC (mg·L$^{-1}$) | Color (Pt–Co units) | Area (ha) | Max. depth (m) | Rel. depth (%) | Transparency (m) | Litt | Wastershed area (ha) | *W/L* | Alt. (m a.s.l.) | Lat. (°,′,″) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.7 | 16.6 | 9.8 | 5.5 | 0.07 | 1.2 | 1.8 | 5 | 7.7 | 8.8 | 2.8 | — | 0.9 | 36 | 5 | 113 | 450149 |
| 1.3 | 44.8 | 44.6 | 1.9 | 0.52 | 0.4 | 0.6 | 3 | 16.2 | 17.7 | 3.9 | 12.4 | 0.7 | 75 | 5 | 461 | 452700 |
| 0.6 | 43.1 | 27.8 | 2.3 | 1.51 | 1.1 | 1.2 | 7 | 42.9 | 18.0 | 2.4 | 10.3 | 0.6 | 379 | 9 | 98 | 435722 |
| 0.6 | 18.7 | 4.3 | 5.6 | 0.75 | 1.6 | 2.0 | 9 | 50.6 | 9.0 | 1.1 | 7.4 | 0.8 | 368 | 7 | 160 | 435722 |
| 1.0 | 41.3 | 16.8 | 9.2 | 1.06 | 3.8 | 4.7 | 29 | 75.0 | 12.8 | 1.3 | 4.7 | 0.4 | 638 | 9 | 484 | 454332 |
| 0.8 | 65.3 | 23.8 | 10.8 | 0.63 | 3.7 | 3.7 | 22 | 2.0 | 7.9 | 5.0 | 4.3 | 0.5 | 43 | 22 | 948 | 450840 |
| 0.8 | 21.8 | 12.3 | 7.3 | 0.69 | 0.8 | 4.2 | 20 | 9.3 | 9.8 | 2.9 | — | 0.8 | 105 | 12 | 449 | 453022 |
| 1.2 | 12.2 | 4.0 | 18.4 | 1.33 | 3.9 | 3.0 | 17 | 25.9 | 9.0 | 1.6 | 5.2 | 0.6 | 245 | 9 | 281 | 470954 |
| 0.7 | 260.2 | 160.0 | 13.5 | 0.81 | 1.3 | 7.7 | 30 | 2.0 | 2.0 | 1.3 | — | 0.9 | 57 | 29 | 880 | 444548 |
| 0.6 | 63.9 | 7.5 | 8.4 | 1.67 | 3.2 | 3.5 | 35 | 43.3 | 22.9 | 3.1 | 4.1 | 0.2 | 482 | 11 | 52 | 441654 |
| 0.6 | 107.0 | 45.8 | 4.7 | 1.55 | 1.8 | 3.5 | 22 | 18.2 | 16.8 | 3.5 | 6.1 | 0.4 | 138 | 8 | 122 | 444843 |
| 0.9 | 47.8 | 24.0 | 4.5 | 2.25 | 1.0 | 5.3 | 12 | 2.0 | 2.8 | 1.8 | — | 1.0 | 150 | 75 | 1034 | 455539 |
| 0.8 | 209.3 | 103.0 | 8.7 | 0.29 | 2.3 | 7.3 | 33 | 2.4 | 7.3 | 4.6 | 4.1 | 0.6 | 23 | 10 | 893 | 445447 |
| 0.6 | 12.6 | 8.0 | 9.2 | 0.93 | 3.4 | 8.0 | 10 | 120.2 | 12.5 | 1.0 | 4.9 | 0.4 | 674 | 6 | 346 | 444156 |
| 0.6 | 40.9 | 31.0 | 2.4 | 1.40 | 0.7 | 1.0 | 7 | 22.3 | 25.3 | 4.6 | 10.7 | 0.4 | 205 | 9 | 67 | 443806 |
| 0.6 | 13.7 | 4.0 | 8.9 | 1.03 | 2.4 | 2.3 | 18 | 768.7 | 15.8 | 0.5 | 6.8 | 0.4 | 3931 | 5 | 87 | 435245 |
| 0.6 | 21.7 | 4.8 | 22.9 | 0.21 | 14.7 | 5.3 | 32 | 5.3 | 12.5 | 5.0 | 2.8 | 0.2 | 27 | 5 | 115 | 450215 |
| 1.3 | 63.3 | 20.3 | 10.1 | 1.52 | 3.1 | 3.5 | 12 | 219.9 | 15.7 | 0.9 | 4.5 | 0.3 | 727 | 3 | 527 | 445246 |
| 0.6 | 7.0 | 3.8 | 5.7 | 1.19 | 1.5 | 0.7 | 9 | 18.2 | 10.7 | 2.2 | 6.5 | 0.6 | 197 | 11 | 143 | 444011 |
| 0.6 | 70.2 | 26.0 | 9.1 | 1.03 | 1.9 | 4.0 | 20 | 16.2 | 11.0 | 2.4 | 4.0 | 0.4 | 160 | 10 | 722 | 445338 |
| 0.7 | 330.3 | 219.7 | 4.2 | 1.48 | 0.5 | 3.7 | 21 | 1.7 | 15.0 | 9.6 | 7.3 | 0.5 | 87 | 51 | 98 | 443750 |
| 0.6 | 10.6 | 5.0 | 5.8 | 0.63 | 2.3 | 2.0 | 14 | 59.5 | 6.1 | 0.7 | — | 0.9 | 273 | 5 | 87 | 450111 |
| 0.6 | 22.3 | 7.8 | 6.6 | 0.15 | 2.2 | 1.8 | 12 | 13.0 | 6.1 | 1.5 | — | 1.0 | 52 | 4 | 78 | 445436 |
| 0.6 | 21.3 | 6.8 | 6.1 | 0.50 | 2.3 | 3.5 | 20 | 170.1 | 17.1 | 1.2 | 6.0 | 0.4 | 1406 | 8 | 189 | 451218 |
| — | 26.4 | 3.8 | — | 1.18 | — | — | — | 4.0 | 10.0 | 4.4 | 5.2 | 0.5 | 13 | 3 | 94 | 443745 |
| 0.4 | 9.7 | 0.8 | 4.9 | 1.32 | 2.2 | 2.5 | 16 | 57.1 | 17.1 | 2.0 | 6.4 | 0.4 | 273 | 5 | 155 | 441201 |
| — | 285.4 | 167.0 | — | 0.32 | — | — | — | 1.0 | 3.5 | 3.1 | — | 1.0 | 4 | 4 | 325 | 442001 |
| 0.6 | 253.6 | 140.0 | 9.8 | 0.87 | 2.2 | 5.0 | 20 | 4.0 | 11.5 | 5.1 | 3.7 | 0.3 | 39 | 10 | 1038 | 443349 |
| 0.7 | 74.5 | 63.3 | 7.6 | 0.34 | 0.5 | 2.0 | 8 | 3.6 | 6.7 | 3.0 | — | 1.0 | 49 | 14 | 814 | 444547 |
| 1.1 | 106.2 | 14.2 | 17.2 | 1.18 | 2.1 | 10.0 | 69 | 42.1 | 13.0 | 1.8 | 2.9 | 0.2 | 237 | 6 | 419 | 461636 |
| 0.7 | 33.0 | 28.5 | 3.6 | 0.18 | 0.6 | 0.2 | 4 | 7.1 | 6.7 | 2.1 | — | 1.0 | 23 | 3 | 141 | 451121 |
| 0.6 | 8.7 | 0.6 | 13.7 | 1.93 | 4.3 | 3.8 | 27 | 13.0 | 11.5 | 2.8 | 3.9 | 0.3 | 109 | 8 | 106 | 450130 |
| 0.9 | 19.3 | 5.9 | 4.8 | 1.51 | 1.1 | 2.0 | 10 | 152.0 | 14.9 | 1.1 | 6.7 | 0.4 | 2178 | 14 | 174 | 442742 |
| — | 209.3 | 156.7 | 25.0 | 1.30 | 1.0 | 3.7 | 42 | 4.0 | 7.9 | 3.5 | 3.3 | 0.4 | 103 | 26 | 408 | 434536 |
| — | 100.0 | 31.7 | 15.8 | 1.20 | 0.5 | 3.3 | 48 | 2.4 | 12.8 | 7.3 | 3.0 | 0.2 | 33 | 14 | 491 | 440627 |
| 16.5 | 166.4 | — | — | — | — | 1.5 | — | 3.1 | 11.0 | 5.5 | 5.5 | 0.5 | 85 | 27 | 1065 | 435300 |
| 1.6 | 41.0 | 28.7 | 9.9 | 0.95 | 2.2 | 1.7 | 12 | 1.0 | 5.8 | 5.1 | — | 0.7 | 9 | 9 | 1009 | 441538 |
| 1.8 | 418.7 | 361.7 | 5.8 | 0.88 | 0.7 | 1.8 | 4 | 4.4 | 8.2 | 3.2 | — | 0.9 | 66 | 15 | 469 | 435413 |
| 1.8 | 66.8 | 51.3 | 7.5 | 0.03 | 1.6 | 0.2 | 4 | 6.9 | 7.5 | 2.5 | — | 0.8 | 61 | 9 | 53 | 424959 |
| — | 534.7 | 236.0 | 34.0 | 2.73 | 0.5 | 5.7 | 95 | 1.8 | 1.2 | 0.8 | — | 1.0 | 163 | 91 | 905 | 441035 |
| 0.4 | 126.9 | 25.3 | 30.4 | 0.74 | 14.7 | 7.7 | 103 | 5.7 | 3.2 | 1.2 | 1.6 | 0.5 | 192 | 32 | 218 | 425436 |
| — | 158.7 | 47.5 | 34.9 | 1.20 | 4.9 | 6.3 | 90 | 2.4 | 5.0 | 2.9 | 1.9 | 0.4 | 228 | 95 | 436 | 435117 |
| — | 233.3 | 184.7 | 17.2 | 0.45 | 1.4 | 2.7 | 28 | 3.6 | 5.7 | 2.7 | 2.8 | 0.5 | 31 | 9 | 472 | 440011 |
| — | 360.3 | 300.0 | 36.5 | 2.05 | 0.4 | 1.7 | 13 | 0.8 | 1.4 | 1.4 | — | 0.9 | 86 | 108 | 791 | 440107 |
| — | 76.7 | 39.0 | 20.2 | 0.86 | 2.5 | 3.7 | 35 | 1.6 | 1.0 | 0.7 | — | 1.0 | 129 | 81 | 610 | 441500 |
| — | 463.0 | 386.7 | 52.7 | 3.95 | 0.5 | 0.3 | 10 | 1.2 | 0.9 | 0.7 | — | 1.0 | 158 | 132 | 664 | 440024 |
| 0.5 | 123.6 | 44.2 | 6.6 | 1.16 | 0.7 | 3.3 | 22 | 3.2 | 17.0 | 8.7 | 6.6 | 0.4 | 171 | 57 | 445 | 435134 |
| 1.1 | 142.0 | 98.2 | 8.0 | 0.32 | 3.2 | 2.5 | 12 | 2.0 | 7.0 | 6.2 | 5.1 | 0.7 | 14 | 7 | 765 | 431825 |
| 1.0 | 100.8 | 74.5 | 12.9 | 0.11 | 3.2 | 3.2 | 18 | 1.2 | 4.5 | 4.0 | — | 0.7 | 28 | 23 | 976 | 443057 |
| — | 553.7 | 461.3 | 32.2 | 1.05 | 0.2 | 1.3 | 23 | 0.8 | 8.0 | 7.9 | — | 1.0 | 158 | 198 | 677 | 440045 |
| 1.2 | 52.1 | 7.3 | 6.9 | 1.01 | 2.0 | 5.5 | 25 | 17.8 | 24.5 | 5.4 | 4.7 | 0.2 | 166 | 9 | 468 | 450014 |
| 1.1 | 214.8 | 133.0 | 12.1 | 0.63 | 2.9 | 4.7 | 29 | 18.1 | 10.0 | 1.9 | 3.1 | 0.3 | 218 | 12 | 802 | 430452 |
| — | 11.5 | — | 34.3 | 1.43 | 4.8 | 3.0 | 38 | 1.5 | 6.4 | 4.6 | 2.6 | 0.4 | 39 | 26 | 381 | 442535 |
| 0.6 | 135.4 | 40.6 | 16.1 | 0.84 | 8.4 | 6.8 | 29 | 6.0 | 6.2 | 2.2 | 2.2 | 0.4 | 26 | 4 | 792 | 431812 |
| 1.7 | 128.8 | 85.5 | 4.7 | 0.35 | 0.2 | 1.5 | 6 | 11.4 | 11.6 | 3.1 | 9.5 | 0.8 | 62 | 5 | 910 | 425500 |

TABLE 1. (*Concluded*)

| | pH | Alk (μeq·L$^{-1}$) | Cond (μmho·cm$^{-1}$) | Na (μeq·L$^{-1}$) | K (μeq·L$^{-1}$) | Mg (μeq·L$^{-1}$) | Ca (μeq·L$^{-1}$) | F (μeq·L$^{-1}$) | Cl (μeq·L$^{-1}$) | Mar-SO$_4$ (μeq·L$^{-1}$) | Non-mar-SO$_4$ (μeq·L$^{-1}$) | NO$_3$ (μeq·L$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 56. Hough P. | 7.91 | 1758.0 | 215.1 | 76.4 | 9.3 | 406.0 | 1824.0 | 4.6 | 33.0 | 3.3 | 346.0 | 3.5 |
| 57. Kings Hill P. | 5.40 | 16.6 | 24.1 | 14.4 | 3.4 | 32.6 | 96.3 | 1.3 | 7.7 | 0.8 | 123.1 | 2.9 |
| 58. Levi P. | 5.42 | 7.5 | 17.5 | 29.8 | 4.0 | 19.4 | 85.0 | 3.5 | 12.0 | 1.2 | 105.8 | 3.8 |
| 59. North King P. | 7.61 | 1156.0 | 118.0 | 29.5 | 11.0 | 133.7 | — | 4.3 | 11.3 | 1.1 | 131.2 | 13.9 |
| 60. Osmore P. | 6.86 | 118.3 | 25.0 | 59.9 | 8.7 | 31.9 | 153.9 | 2.3 | 15.8 | 1.6 | 100.4 | 3.0 |
| 61. Pigeon P. | 6.62 | 90.0 | 28.4 | 37.1 | 6.6 | 36.2 | 163.1 | 1.0 | 10.8 | 1.1 | 112.3 | 5.5 |
| 62. Walton P. | 7.59 | 738.5 | 79.3 | 34.8 | 6.6 | 116.2 | 713.9 | 2.8 | 16.3 | 1.6 | 96.9 | 5.5 |
| 63. Westford P. | 7.15 | 454.0 | 68.4 | 33.6 | 6.7 | 198.1 | 409.9 | 1.6 | 11.4 | 1.1 | 133.3 | 1.3 |

surface sediment, the cores supplied deeper sediment for paleolimnological studies (Davis et al. 1980, 1983, 1990; Norton et al. 1985). All corers had clear acrylic core tubes, making it possible to reject cores with a disturbed sediment/water interface. Detailed coring and core-sectioning procedures are given in Davis (1986).

In the laboratory, 0.50 mL of surface sediment from each lake was heated in chromic acid to digest organic matter. After a series of distilled water rinses, an aliquot of the residue was used to prepare microscope slides by the settling chamber method of Battarbee (1973). Coverslips with the dried samples were mounted in Hyrax or Naphrax resin for light microscope observations at 400–2000 magnifications. Whole-diatom valves and fragments greater than one-half valve were identified to as precise a taxonomic level as possible. At least 600 valves were identified and counted per sample, except for samples from 14 New Hampshire lakes where at least 500 valves were identified and counted. Additional procedural details are given in Davis and Anderson (1986), Kingston (1986), and Ford (1990). Taxonomy followed PIRLA standards (Camburn et al. 1984–86; Kingston 1986).

### Data Base and Statistical Methods

The 29 chemical and physical variables (Table 1) include five derived variables: (1) littoral index (=litt), defined as the ratio of transparency to maximum depth, (2) watershed/lake area ratio (*W/L*), (3) relative depth ($z_r$; Wetzel 1983), (4) marine SO$_4$ (1 μeq mar-SO$_4$ = 0.1 μeq Cl), and (5) non-mar-SO$_4$ (=total SO$_4$ − mar-SO$_4$). The mean value for each variable at each lake was used in the statistical analyses.

To define the strongest gradients in the environmental data set, principal components analyses (PCA) of 26 chemical/physical variables were done using Biomedical Computer Programs (BMD) P-series, program 4M (Dixon and Brown 1979). For PCA, values except for pH and latitude were log-transformed, as some of the distributions were strongly skewed (e.g., alk), and a Pearson correlation matrix was used with varimax rotation. Ammonium was not included in the PCA because values were lacking for 15 lakes. Transparency was not included because transparency exceeded maximum water depth in 18 lakes. Catchment area was not included because it was considered redundant with *W/L* and lake area.

In statistical analyses of the diatom data set, we used only the 187 taxa that occurred in three or more lakes (a total of 594 taxa were identified; complete diatom data are available from author D.S.A.). To decipher the relationships among the 63 diatom samples, and to provide a basis for a first set of inferential models, we carried out detrended correspondence analyses (DCA) using DECORANA of Hill (1979). Diatom counts were first subjected to a log$_2$-transformation because preliminary application of DCA models calibrated with nontransformed data resulted in excessively "noisy" pH and alk reconstructions (Davis et al. 1990, 1994).

We tested the suitability of the derived DCA axes for development of models to infer environmental conditions by carrying out an indirect gradient analysis. The analysis consisted of Spearman (rank-order) and Pearson correlations between (1) the DCA sample scores on each of the first two DCA axes and (2) the lake values of the 29 environmental variables.

Cluster analyses of the diatom taxa were performed and clusters were plotted on the pH and alk gradients to detect major patterns of distribution on those gradients. We used an agglomerative average-link clustering routine with a Pearson correlation coefficient similarity measure (BMD program 1M). The raw diatom percentages (187 taxa) were not used because many of the taxa had very noisy distributions. We removed some of the noise (non-pH or non-alk variance) by ordering the 63 samples according to pH and calculating two-point running means, producing 62 means for each taxon as input to the clustering routine. A parallel procedure was used to obtain taxa clusters in relation to alk. A major goal was to obtain clusters that would provide an effective basis for a second set of inferential models of pH and alk.

The final approach for exploring relationships between the biological and environmental data consisted of canonical correspondence analysis (CCA) of the combined data sets using CANOCO of ter Braak (1987). This form of direct gradient analysis provided the basis for the third set of inferential models. The environmental variables were the same as for PCA, but as in the CCA by Stevenson et al. (1991) the data were not transformed, except for a separate CCA with log$_{10}$ alk. To accommodate the lowest alk (−39.7 μeq·L$^{-1}$)

| $NH_4$ (μeq·L$^{-1}$) | Total Al (μg·L$^{-1}$) | Exch Al (μg·L$^{-1}$) | Total P (μg·L$^{-1}$) | $SiO_2$ (mg·L$^{-1}$) | Chl *a* (μg·L$^{-1}$) | DOC (mg·L$^{-1}$) | Color (Pt–Co units) | Area (ha) | Max. depth (m) | Rel. depth (%) | Transparency (m) | Litt | Wastershed area (ha) | *W/L* | Alt. (m a.s.l.) | Lat. (°,′,″) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.4 | 2.7 | 0.8 | 10.8 | 2.42 | 3.2 | 4.0 | 19 | 12.7 | 14.5 | 3.6 | 5.3 | 0.4 | 56 | 4 | 237 | 434703 |
| 10.5 | 187.3 | 34.5 | 16.4 | 0.68 | 6.8 | 3.2 | 29 | 2.4 | 3.8 | 2.2 | 2.2 | 0.6 | 176 | 73 | 335 | 444414 |
| — | 84.0 | 39.0 | 9.7 | 0.46 | 2.0 | 4.0 | 10 | 8.9 | 6.8 | 2.0 | 3.4 | 0.5 | 126 | 14 | 497 | 441559 |
| — | 5.7 | 2.5 | 75.9 | 2.39 | 3.5 | 2.7 | 25 | 0.8 | 4.3 | 4.3 | 3.3 | 0.8 | 19 | 24 | 369 | 442450 |
| — | 23.0 | 10.5 | 61.3 | 3.79 | 4.4 | 2.7 | 23 | 19.4 | 3.0 | 0.6 | 2.5 | 0.8 | 446 | 23 | 448 | 441828 |
| 0.6 | 60.9 | 22.5 | 11.2 | 1.81 | 3.9 | 4.0 | 21 | 24.0 | 6.5 | 1.2 | 3.9 | 0.6 | 341 | 14 | 595 | 441449 |
| — | 19.3 | 7.0 | 19.7 | 1.01 | 0.9 | 2.3 | 23 | 3.5 | 7.9 | 3.7 | 4.5 | 0.6 | 58 | 17 | 400 | 442641 |
| 0.8 | 13.0 | 6.8 | 13.9 | 1.30 | 10.2 | 3.2 | 17 | 4.1 | 10.0 | 4.4 | 2.6 | 0.3 | 147 | 36 | 241 | 443535 |

TABLE 2. Summary of characteristics of the 63-lake data set, based largely on three to eight samples of the mixed part of water column over the deepest part of the lake, widely spaced throughout ice-free seasons 1983–84, supplemented by additional samples for 1978–84 for some lakes (see Materials and Methods). Variables lacking units are μeq·L$^{-1}$ except for pH.

| Variable | *N* | Mean | SD | Median | Minimum | Maximum |
|---|---|---|---|---|---|---|
| pH | 63 | 5.95 | 0.90 | 5.99 | 4.45 | 7.91 |
| Alk | 63 | 141.2 | 346.7 | 26.7 | −39.7 | 1758.0 |
| Cond (μmho·cm$^{-1}$) | 63 | 32.5 | 33.9 | 23.0 | 12.2 | 215.1 |
| Na | 63 | 47.5 | 34.1 | 37.1 | 9.1 | 229.2 |
| K | 63 | 7.6 | 3.8 | 6.7 | 1.9 | 22.8 |
| Mg | 63 | 45.9 | 61.3 | 30.2 | 4.3 | 406.0 |
| Ca | 62 | 160.2 | 284.1 | 81.4 | 12.8 | 1824.0 |
| F | 62 | 6.3 | 15.2 | 1.8 | 0.3 | 84.3 |
| Cl | 63 | 25.2 | 23.7 | 14.5 | 5.0 | 138.3 |
| Mar-$SO_4$ | 63 | 2.5 | 2.4 | 1.4 | 0.5 | 13.8 |
| Non-mar-$SO_4$ | 63 | 87.5 | 40.3 | 82.6 | 34.8 | 346.0 |
| $NO_3$ | 63 | 2.9 | 3.3 | 1.8 | 0.1 | 17.0 |
| $NH_4$ | 47 | 1.4 | 2.7 | 0.7 | 0.4 | 16.5 |
| Total Al (μg·L$^{-1}$) | 63 | 114.2 | 132.1 | 63.9 | 2.7 | 553.7 |
| Exch Al (μg·L$^{-1}$) | 61 | 68.2 | 101.3 | 27.8 | 0.6 | 461.3 |
| Total P (μg·L$^{-1}$) | 60 | 14.9 | 14.4 | 9.8 | 1.9 | 75.9 |
| $SiO_2$ (mg·L$^{-1}$) | 62 | 1.1 | 0.8 | 1.0 | 0.1 | 4.0 |
| Chl *a* (μg·L$^{-1}$) | 60 | 2.8 | 2.9 | 2.2 | 0.2 | 14.7 |
| DOC (mg·L$^{-1}$) | 61 | 3.4 | 2.1 | 3.2 | 0.2 | 10.0 |
| Color (Pt–Co units) | 60 | 23.8 | 20.6 | 20.0 | 3.0 | 103.0 |
| Area (ha) | 63 | 34.4 | 102.8 | 6.0 | 0.8 | 768.0 |
| Max. depth (m) | 63 | 9.7 | 5.6 | 8.8 | 0.9 | 24.5 |
| Rel. depth (%) | 63 | 3.2 | 2.2 | 3.0 | 1.0 | 13.0 |
| Transparency (m) | 45[a] | 4.9 | 2.4 | 4.5 | 1.6 | 12.4 |
| Litt[b] | 63 | 0.6 | 0.2 | 0.5 | 0.2 | 1.0 |
| Watershed area (ha) | 63 | 269.2 | 577.6 | 126.0 | 4.0 | 3931.0 |
| *W/L* | 63 | 25.4 | 35.8 | 11.0 | 3.0 | 198.0 |
| Alt. (m a.s.l.) | 63 | 455.4 | 311.0 | 436.0 | 52.0 | 1123.0 |
| Lat. north | 63 | 44°27′46″ | — | 44°26′41″ | 42°49′59″ | 47°09′54″ |

[a] Eighteen lakes had Secchi transparency greater than maximum depth and are not included here.

[b] Littoral index is the ratio of transparency to maximum depth. Lakes in which the disk was visible on the lake bottom have a maximum littoral index of 1.0.

in the transformation, 40.7 was added to all alk values. The diatom data inputs consisted of the 63 percentage counts (187 taxa).

### Calibration of Inferential Models

Calibrations were carried out for three inferential models of pH and three of alk: (1) DCA simple regression model,

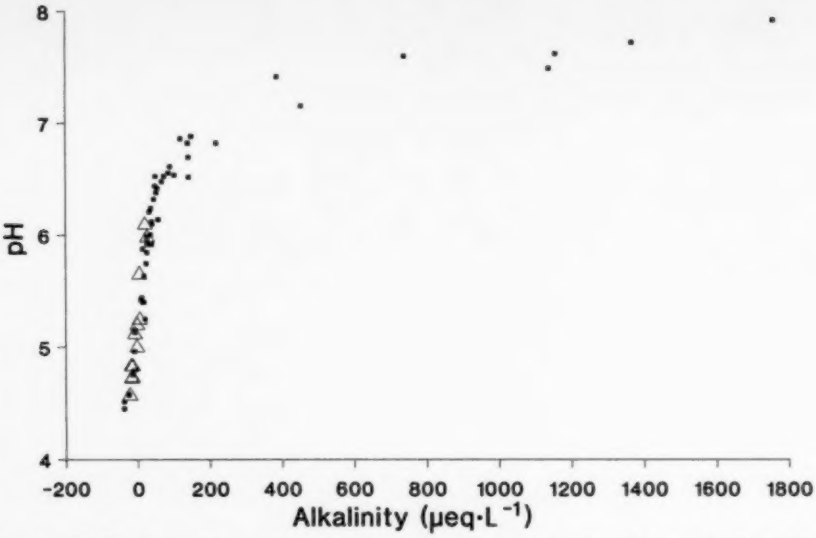

FIG. 2. pH/alk relationships of 63 study lakes. The 12 reconstruction lakes are indicated by triangles.

(2) CLUSTER (CLUS) multiple regression model, and (3) CCA simple regression model. For each model, calibrations were run for full (63-lake) and culled (56-lake) data sets. Because of the skewed distribution of alk, we repeated the alk calibrations using $\log_{10}$-transformed alks.

*DCA simple regression model*

The diatom sample scores (derived by WA) on the first DCA axis were regressed on pH and on alk. As explained in the results, axis 1 is strongly correlated with pH and alk.

*CLUS multiple regression model*

For pH, 11 clusters were defined at 55% similarity, a point of "stability" in the dendrogram. Only seven of these clusters are strongly related (linearly) to pH or contain taxa abundantly represented in any of the cores from the 12 reconstruction lakes. These seven contain over 85% of the tallied diatoms. Only three samples contain fewer than 60% of their diatoms in these clusters. The seven clusters were used in multiple linear regressions on pH. For alk, cluster analysis again produced 11 clusters at a point of "stability" (57% similarity). Nine of these are strongly related to alk or contain taxa that are abundant in one or more of the cores. These nine are used in multiple regressions on alk. The nine alk clusters contain an even higher percentage of the total diatoms than the seven pH clusters.

*CCA simple regression model*

The same CCA program (CANOCO of ter Braak (1987)) used for direct gradient analysis provided an indirect means of implementing a WA calibration with classical linear deshrinking (ter Braak and van Dam 1989). This is effectively the same method used by Stevenson et al. (1989, 1991) and Birks et al. (1990). The generalized calibration equation is

$$\mathrm{IE} = \mathrm{MM} + \frac{(\mathrm{SDM} \times \mathrm{Axis\ 1\ BAS})}{\mathrm{CC}}$$

in which axis 1 is constrained to the environmental variable of interest, IE = inferred value for environmental variable, MM = mean of measured values of environmental variable in the data set, SDM = standard deviation of measured values of the environmental variable in the data set, Axis 1 BAS = bioindicator assemblage score on CCA axis 1 (e.g., a down-core diatom assemblage used to infer a value for the environmental variable), and CC = canonical coefficient for standardized environmental variable in the data set.

### Culling the 63-Lake Data Set

The objective of culling was to improve the accuracy of inferences at our acidic reconstruction lakes. As explained earlier, it is highly unlikely that the reconstruction lakes could have had $\mathrm{pH} > 6.5$ and $\mathrm{alk} > 100\ \mu\mathrm{eq}\cdot\mathrm{L}^{-1}$ in the period of reconstruction. Three criteria were used for selecting sites for culling: (1) the diatom assemblage bears little or no resemblance to those from calibration lakes with measured $\mathrm{pH} < 6.5$ and $\mathrm{alk} < 100\ \mu\mathrm{eq}\cdot\mathrm{L}^{-1}$ and to down-core assemblages in the reconstruction lakes, (2) culling of the site decreases residuals at sites with measured $\mathrm{pH} < 6.5$ and alk $< 100\ \mu\mathrm{eq}\cdot\mathrm{L}^{-1}$, and (3) the site's regression-inferred pH or alk deviates from the measured value by >1.75 of the root mean square error (RMSE). This approach, largely directed at a particular set of reconstruction lakes, differs from the more general approach of Birks et al. (1990) in their identification of "rogue samples".

## Results, Part 1. Lakes and Their Diatoms, and Gradient Analyses

### Distributions of Chemical and Physical Characteristics

The distribution of lake area is strongly skewed (med. 6.0, mean 34.4 ha) because five lakes are larger than 100 ha (max. 768 ha) (Table 2). The near-even distribution of pH imposed by the lake selection process results in similar median (5.99) and mean (5.95) pH values, but such similarity is not true of alk: the difference between the median of 27 $\mu\mathrm{eq}\cdot\mathrm{L}^{-1}$ and the mean of 141 $\mu\mathrm{eq}\cdot\mathrm{L}^{-1}$ results from a small number of lakes with much higher alks, the seven highest ranging from 386 to 1758 $\mu\mathrm{eq}\cdot\mathrm{L}^{-1}$ (Table 1; Fig. 2). Calcium and, to a lesser degree, Mg are correlated with alk:

TABLE 3. Rotated factor loadings for the five axes extracted by the PCA of 26 environmental variables for the full ($N = 63$) and culled ($N = 56$) data sets.

| Variable | $N = 63$ axes |  |  |  |  | $N = 56$ axes |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| pH | 0.878 | 0.086 | 0.259 | 0.125 | 0.126 | 0.864 | 0.362 | 0.132 | 0.164 | 0.055 |
| Alk | 0.872 | 0.009 | 0.292 | 0.167 | 0.087 | 0.765 | 0.429 | 0.010 | 0.215 | 0.094 |
| Cond | 0.865 | 0.170 | 0.105 | 0.067 | −0.044 | 0.250 | 0.148 | 0.408 | 0.129 | 0.697 |
| Na | 0.158 | 0.866 | 0.279 | 0.029 | 0.046 | 0.158 | 0.270 | 0.880 | 0.015 | 0.114 |
| K | 0.207 | 0.342 | 0.632 | 0.189 | 0.033 | 0.189 | 0.633 | 0.350 | 0.186 | 0.133 |
| Mg | 0.891 | 0.094 | −0.024 | 0.139 | 0.141 | 0.589 | −0.055 | 0.145 | 0.208 | 0.566 |
| Ca | 0.898 | −0.058 | 0.341 | 0.163 | 0.036 | 0.596 | 0.556 | −0.078 | 0.253 | 0.431 |
| F | −0.045 | 0.005 | 0.881 | −0.045 | −0.134 | −0.160 | 0.881 | 0.013 | −0.082 | 0.021 |
| Cl | 0.068 | 0.952 | −0.080 | −0.150 | 0.086 | 0.073 | −0.104 | 0.946 | −0.156 | 0.050 |
| Mar-$SO_4$ | 0.068 | 0.952 | −0.080 | −0.150 | 0.086 | 0.073 | −0.104 | 0.946 | −0.156 | 0.050 |
| Non-mar-$SO_4$ | 0.404 | −0.257 | 0.304 | −0.104 | 0.069 | −0.178 | 0.318 | −0.305 | −0.122 | 0.801 |
| $NO_3$ | 0.183 | −0.403 | 0.702 | −0.101 | −0.225 | −0.077 | 0.738 | −0.427 | −0.123 | 0.015 |
| Total Al | −0.768 | −0.250 | 0.296 | 0.122 | −0.089 | −0.780 | 0.336 | −0.304 | 0.108 | 0.225 |
| Exch Al | −0.747 | −0.285 | 0.272 | −0.109 | −0.176 | −0.809 | 0.285 | −0.313 | −0.156 | 0.158 |
| Total P | 0.217 | −0.254 | 0.459 | 0.509 | −0.487 | −0.196 | 0.534 | −0.228 | 0.562 | 0.070 |
| $SiO_2$ | 0.385 | 0.027 | 0.734 | 0.069 | 0.009 | 0.245 | 0.749 | 0.038 | 0.026 | 0.193 |
| Chl *a* | 0.348 | 0.029 | −0.369 | 0.725 | −0.113 | 0.168 | −0.319 | 0.048 | 0.795 | 0.072 |
| DOC | 0.062 | −0.295 | −0.016 | 0.807 | 0.166 | 0.138 | −0.006 | −0.320 | 0.793 | −0.021 |
| Color | 0.092 | −0.048 | 0.320 | 0.869 | −0.062 | −0.029 | 0.358 | −0.058 | 0.863 | 0.028 |
| Area | 0.198 | 0.227 | −0.154 | −0.055 | 0.581 | 0.767 | −0.252 | 0.230 | −0.058 | 0.243 |
| Max. depth | 0.189 | 0.209 | −0.226 | −0.087 | 0.891 | 0.411 | −0.306 | 0.204 | −0.104 | 0.141 |
| Rel. depth | −0.018 | −0.039 | −0.078 | −0.028 | 0.250 | −0.431 | −0.058 | −0.039 | −0.042 | −0.116 |
| Litt | −0.220 | −0.026 | −0.017 | −0.552 | −0.736 | −0.313 | 0.038 | −0.022 | −0.525 | −0.181 |
| *W/L* | −0.149 | −0.247 | 0.589 | 0.164 | −0.440 | −0.412 | 0.668 | −0.232 | 0.123 | 0.034 |
| Alt. | −0.194 | −0.817 | 0.196 | 0.097 | −0.169 | −0.260 | 0.211 | −0.822 | 0.102 | 0.071 |
| Lat. | 0.244 | −0.184 | −0.049 | −0.036 | 0.098 | 0.511 | 0.019 | −0.231 | −0.075 | −0.482 |
| Variance explained (%) | 22.6 | 15.8 | 14.4 | 10.7 | 9.3 | 19.9 | 17.3 | 16.9 | 11.5 | 7.6 |

Pearson correlation coefficients are 0.98 and 0.90, respectively. In our data set, lakes with high sum of base cations typically occur at low altitudes and have large surface areas.

Aluminum concentrations are commonly inversely related to pH in limnological surveys in New England (Haines and Akielaszek 1983; Linthurst et al. 1986). This relationship is true for our lakes, but the linear $r^2$ of pH with total Al is only 0.26 and with exch Al only 0.21. The relationships are better approximated by quadratic equations, with $r^2$ of 0.52 and 0.55, respectively, because of the stoichiometry of water–Al reactions below pH ~5.5. The relationship between Al and pH is weakened by the inclusion of three New Hampshire lakes (40, 46, and 50 in Table 1) with particularly high Al at intermediate pH. These lakes on the Conway Granite have the highest concentrations of F, and their outlier positions for Al probably result, at least in part, from Al–F complexation. Additional lake physical and chemical characteristics are summarized in Table 2.

## Principal Components Analysis (PCA) of Chemical and Physical Variables

The PCA (Table 3) indicates the strongest chemical and physical gradients in the 63-lake data set. Component 1, accounting for 22.6% of the variance (%var), has heavy positive loadings of pH, alk, Ca, Mg, and conductance (cond) and heavy negative loadings of both Al variables. This component is largely a function of geological and edaphic conditions. Component 2 (15.8%var) indicates marine influence and altitude. Sodium, Cl, and mar-$SO_4$ have heavy positive loadings, and altitude has a heavy negative loading. The high-altitude sites are generally far inland and thus receive less marine aerosol. Component 3 (14.4%var) reflects the special geological condition at some New Hampshire lakes (Norton et al. 1989), with heavy loadings for F, $SiO_2$, $NO_3$, K, and the *W/L* ratio. These lakes occur on the Conway

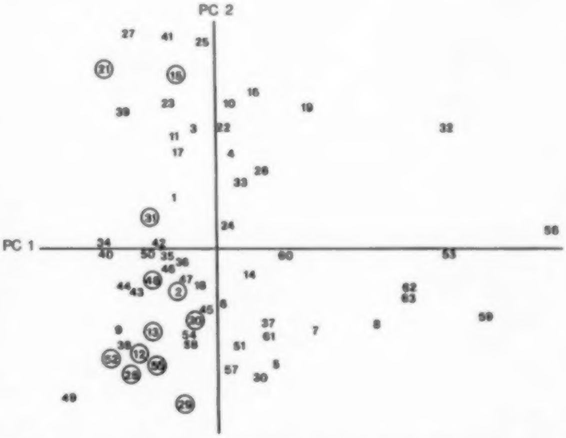

FIG. 3. Ordination of the 63 lakes on principal components 1 and 2 based on 26 environmental variables. Names and characteristics of numbered lakes are given in Table 1. Circled numbers indicate lakes for which paleolimnological reconstructions have been carried out (Davis et al. 1983, 1990, 1994; Norton et al. 1985).

TABLE 4. The 113 taxa mentioned in this paper and by Davis et al. (1990, 1994) in paleolimnological reconstructions based on the results of this paper, and the code for taxa plotted in Fig. 6b. The number of lakes of occurrence, the abundance-weighted mean (AWM) pH and alk, and the membership in taxa clusters (Fig. 5) are given for each taxon. Taxa cluster 8 for pH is not included in regressions. Taxa are listed alphabetically in four groups: centrics, araphes, monoraphes, and biraphes, in order.

| Taxon | Code | *n* | pH | | Alkalinity | |
|---|---|---|---|---|---|---|
| | | | AWM | cluster | AWM | cluster |
| *Cyclotella comta* (Ehr.) Kütz | Cc | 26 | 6.95 | 1 | 424.2 | 1 |
| *Cyclotella michiganiana* Skvortz. | Cm | 15 | 7.21 | 1 | 670.3 | 1 |
| *Clyclotella stelligera* Cl. et Grun. | Cs | 37 | 6.56 | 1 | 145.5 | 1 |
| Other *Cyclotella* | | 11 | 6.81 | 1 | 398.2 | 1 |
| *Melosira ambigua* (Grun.) O. Mull. | | 24 | 6.74 | 1 | 248.0 | 1 |
| *Melosira distans* (Ehr.) Kütz. | Md | 28 | 5.75 | 6 | 31.7 | 7 |
| *Melosira distans* var. *nivalis* (W. Sm.) Kirchner | Mdn | 20 | 5.47 | 4 | 8.9 | 9 |
| *Melosira distans* var. *nivaloides* Camburn | Mdv | 30 | 6.05 | 4 | 44.7 | 4 |
| *Melosira distans* var. *tenella* (Nygaard) Florin | Mdt | 12 | 6.08 | 3 | 45.4 | 3 |
| *Melosira italica* ssp. *subarctica* O. Mull. | Mi | 16 | 6.56 | 1 | 101.9 | 1 |
| *Melosira lirata* (Ehr.) Kütz. | Ml | 34 | 5.60 | 6 | 16.8 | 9 |
| *Melosira nygaardii* Camburn | Mn | 17 | 5.59 | 2 | 19.8 | 9 |
| *Melosira perglabra* Østr. | Mp | 33 | 5.60 | 6 | 19.1 | 9 |
| *Melosira perglabra* var. *floriniae* Camburn | Mpf | 31 | 5.71 | 4 | 20.4 | 4 |
| *Asterionella formosa* Haas. | Af | 23 | 6.94 | 1 | 348.1 | 1 |
| *Asterionella ralfsii* var. *americana* Körn. (>45 μm) | Arl | 11 | 5.53 | 2 | 13.4 | 2 |
| *Asterionella ralfsii* var. *americana* Körn. (<45 μm) | Ars | 8 | 4.56 | 7 | −34.9 | 8 |
| *Fragilaria acidobiontica* Charles | Fa | 6 | 5.12 | 5 | 11.1 | 4 |
| *Fragilaria brevistriata* Grun. | | 14 | 6.86 | 3 | 476.7 | 3 |
| *Fragilaria constricta* Ehr. | Fs | 26 | 5.19 | 7 | 15.8 | 9 |
| *Fragilaria construens* (Ehr.) Grun. | Fc | 21 | 7.34 | 1 | 820.0 | 1 |
| *Fragilaria construens* var. *subsalina* Hust. | | 7 | 7.19 | 1 | 776.4 | 1 |
| *Fragilaria construens* var. *venter* (Ehr.) Grun. | Fcv | 33 | 6.72 | 1 | 322.5 | 1 |
| *Fragilaria crotonensis* Kitton | Fo | 11 | 7.75 | 1 | 1470.4 | 1 |
| *Fragilaria hungarica* var. *tumida* Cl.-Eul. | | 8 | 5.41 | 5 | 11.5 | 8 |
| *Fragilaria pinnata* Ehr. | Fp | 32 | 7.04 | 1 | 620.6 | 1 |
| *Fragilaria virescens* var. *exigua* Grun. | Fv | 37 | 6.00 | 5 | 55.3 | 2 |
| *Synedra filiformis* var. *exilis* Cl.-Eul. | | 16 | 6.50 | 1 | 100.9 | 1 |
| *Synedra minuscula* Grun. | | 8 | 6.67 | 1 | 430.4 | 1 |
| *Synedra nana* Meist. | | 10 | 6.35 | 2 | 120.8 | 2 |
| *Synedra radians* Kütz. | | 6 | 7.63 | 1 | 1383.3 | 1 |
| *Synedra rumpens* Kütz. | | 17 | 6.91 | 1 | 460.0 | 1 |
| *Synedra* sp. 2 PIRLA[a] | S2 | 18 | 6.86 | 3 | 440.4 | 3 |
| Other *Synedra* | | 29 | 6.66 | 1 | 358.9 | 1 |
| *Tabellaria binalis* (Ehr.?) Grun. | Tb | 10 | 4.64 | 7 | −24.6 | 9 |
| *Tabellaria quadriseptata* Knuds. | Tp | 28 | 4.98 | 7 | −5.6 | 9 |
| *Tabellaria flocculosa* (Roth) Kütz., strain III, sensu Koppen | T3 | 44 | 5.72 | 5 | 34.7 | 6 |
| *Tabellaria flocculosa* (Roth) Kütz., strain IIIp, sensu Koppen | T3P | 21 | 6.33 | 2 | 76.1 | 2 |
| *Tabellaria flocculosa* (Roth) Kütz., strain IV, sensu Koppen | | 26 | 5.97 | 2 | 54.4 | 5 |
| *Tabellaria flocculosa* var. *linearis* (Roth) Kütz. | | 17 | 6.10 | 3 | 84.7 | 2 |
| *Achnanthes austriaca* var. *helvetica* Hust. | Aa | 10 | 5.22 | 6 | 12.6 | 9 |
| *Achnanthes didyma* Hust. | | 11 | 6.78 | 1 | 2166.3 | 1 |
| *Achnanthes exigua* Grun. | | 5 | 7.51 | 1 | 931.9 | 1 |
| *Achnanthes lanceolata* var. *dubia* Grun. | | 6 | 7.56 | 1 | 1130.8 | 1 |
| *Achnanthes levanderi* Hust. | Al | 29 | 6.28 | 4 | 117.7 | 4 |
| *Achnanthes linearis* (W. Sm.) Grun. | | 19 | 6.65 | 1 | 351.7 | 1 |
| *Achnanthes linearis* f. *curta* H. L. Sm. | | 11 | 6.83 | 1 | 372.0 | 1 |
| *Achnanthes linearis* var. *pusilla* Grun. | | 7 | 6.60 | 1 | 241.4 | 1 |
| *Achnanthes marginulata* Grun. | Am | 46 | 5.82 | 8 | 32.4 | 5 |
| *Achnanthes minutissima* Kütz. | Ai | 31 | 6.91 | 1 | 506.0 | 1 |
| *Achnanthes suchlandtii* Hust. | | 5 | 6.84 | 1 | 264.4 | 1 |
| Other *Achnanthes* | | 41 | 6.79 | 1 | 492.4 | 1 |
| All *Cocconeis* | | 10 | 6.93 | 1 | 512.4 | 1 |
| *Actinella punctata* Lewis | Ap | 23 | 5.12 | 7 | −1.9 | 9 |
| *Amphora ovalis* (Kütz.) Kütz. | | 9 | 6.72 | 3 | 268.4 | 3 |
| *Amphora perpusilla* (Grun.) Grun. | | 7 | 7.23 | 1 | 770.9 | 1 |

TABLE 4. (*Concluded*)

| Taxon | Code | *n* | pH | | Alkalinity | |
|---|---|---|---|---|---|---|
| | | | AWM | cluster | AWM | cluster |
| Other *Amphora* | | 7 | 7.19 | 1 | 735.1 | 1 |
| *Anomoeoneis serians* (Bréb. ex Kütz.) Cl. | As | 9 | 5.05 | 7 | −10.2 | 9 |
| *Anomoeoneis serians* var. *brachysira* (Bred. ex Kütz.) Hust. | Asb | 49 | 5.77 | 5 | 25.0 | 6 |
| *Anomoeoneis vitrea* (Grun.) Ross | Av | 38 | 6.18 | 8 | 97.9 | 5 |
| *Cymbella hebridica* Grun. ex Cl. | Ch | 30 | 5.44 | 8 | 10.0 | 5 |
| *Cymbella lunata* W. Sm. | Cu | 27 | 5.98 | 5 | 39.5 | 6 |
| *Cymbella microcephala* Grun. | | 14 | 7.15 | 1 | 788.3 | 1 |
| *Cymbella* sp. 1 PIRLA[a] | Cl | 38 | 5.49 | 5 | 10.9 | 8 |
| *Eunotia bidentula* W. Sm. | Eb | 15 | 4.74 | 7 | −18.8 | 9 |
| *Eunotia carolina* Patr. | | 9 | 4.86 | 7 | −17.8 | 8 |
| *Eunotia curvata* (Kütz.) Largerst. | Ec | 44 | 5.28 | 7 | 6.8 | 9 |
| *Eunotia exigua* (Bréb. ex. Kütz.) Rahb. | Ee | 33 | 5.61 | 5 | 17.9 | 5 |
| *Eunotia flexuosa* Bréb. ex Kütz. | | 27 | 5.47 | 7 | 14.4 | 9 |
| *Eunotia incisa* W. Sm. ex Greg. | Ei | 41 | 5.45 | 7 | 16.0 | 9 |
| *Eunotia pectinalis* (O. Mull. ?) Rabh. | Ep | 34 | 5.52 | 7 | 19.0 | 6 |
| *Eunotia pectinalis* var. *minor* (Kütz.) Rabh. | Epm | 42 | 5.00 | 7 | −4.4 | 9 |
| *Eunotia pectinalis* var. *ventricosa* Grun. | Epv | 8 | 5.59 | 2 | 20.2 | 5 |
| *Eunotia serra* Ehr. | | 15 | 5.22 | 7 | 3.7 | 8 |
| *Eunotia tenella* (Grun.) Cl. | | 34 | 5.20 | 7 | −0.5 | 9 |
| *Eunotia vanheurckii* var. *intermedia* (Krasske ex Hust.) Patr. | | 29 | 5.21 | 7 | −1.6 | 8 |
| Other *Eunotia* | | 51 | 5.26 | 7 | 4.0 | 9 |
| *Frustulia rhomboides* (Ehr.) DeT. | | 42 | 5.37 | 7 | 7.2 | 9 |
| *Frustilia rhomboides* var. *saxonica* (Rabh.) DeT. | Frs | 44 | 5.32 | 7 | 4.7 | 9 |
| *Frustulia* cf. *magaliesmontana* Cholnoky | Fm | 30 | 5.08 | 7 | −6.0 | 9 |
| *Navicula bremensis* Hust. | | 7 | 4.90 | 7 | −11.7 | 8 |
| *Navicula hassiaca* Krasske | Nh | 12 | 5.84 | 5 | 26.8 | 2 |
| *Navicula leptostriata* Jørg. | Nl | 43 | 5.65 | 5 | 19.8 | 7 |
| *Navicula mediocris* Krasske | Nm | 35 | 5.58 | 5 | 19.2 | 5 |
| *Navicula minima* Grun. | | 24 | 6.35 | 1 | 228.4 | 1 |
| *Navicula pseudoscutiformis* Hust. | | 12 | 6.70 | 1 | 324.6 | 1 |
| *Navicula pupula* Kütz. | | 20 | 6.81 | 1 | 439.0 | 1 |
| *Navicula radiosa* Kütz. | | 13 | 6.79 | 1 | 382.9 | 1 |
| *Navicula radiosa* var. *tenella* (Bréb. ex Kütz.) Grun. | | 9 | 7.15 | 1 | 683.3 | 1 |
| *Navicula seminuloides* Hust. | Ni | 34 | 6.45 | 1 | 211.6 | 1 |
| *Navicula subrotundata* Hust. | | 5 | 6.68 | 1 | 108.0 | 1 |
| *Navicula subtilissima* Cl. | Ns | 38 | 5.18 | 7 | −2.4 | 9 |
| *Navicula tenuicephala* Hust. | Nt | 12 | 5.22 | 5 | −3.4 | 8 |
| *Navicula* sp. 4 PIRLA[a] | | 11 | 6.83 | 1 | 244.0 | 1 |
| Other *Navicula* | | 50 | 6.50 | 1 | 283.7 | 1 |
| *Neidium affine* (Ehr.) Pfitz. | Na | 36 | 5.09 | 7 | −4.6 | 9 |
| *Neidium bisulcatum* (Lagerst.) Cl. | Nb | 17 | 4.96 | 7 | −12.1 | 9 |
| *Neidium iridis* (Ehr.) Cl. | | 15 | 5.11 | 7 | −5.4 | 9 |
| *Neidium iridis* var. *amphigomphus* (Ehr.) A. Mayer | Nia | 25 | 5.07 | 7 | −5.1 | 9 |
| *Neidium* sp. 2 PIRLA[a] | | 10 | 4.79 | 7 | −23.5 | 8 |
| *Nitzschia* sp. 1 PIRLA[a] | Nl | 37 | 6.09 | 5 | 91.2 | 2 |
| Other *Nitzschia* | | 46 | 6.24 | 1 | 221.6 | 1 |
| *Pinnularia abaujensis* var. *rostrata* (Patr.) Patr. | | 16 | 5.55 | 5 | 59.9 | 7 |
| *Pinnularia abaujensis* var. 2 PIRLA[a] | P2 | 25 | 5.19 | 7 | 7.3 | 9 |
| *Pinnularia biceps* Greg. | Pb | 34 | 5.96 | 8 | 83.0 | 5 |
| *Pinnularia microstauron* (Ehr.) Cl. | | 21 | 4.89 | 7 | −14.3 | 9 |
| *Pinnularia* sp. 7 PIRLA[a] | | 8 | 5.09 | 7 | −3.7 | 9 |
| *Pinnularia* sp. 11 PIRLA[a] | | 8 | 5.04 | 7 | −4.5 | 9 |
| Other *Pinnularia* | | 55 | 5.57 | 8 | 33.8 | 5 |
| *Semiorbis hemicyclus* (Ehr.) Patr. | Sh | 23 | 5.32 | 7 | 2.2 | 9 |
| *Stauroneis anceps* f. *gracilis* Rabh. | Sa | 31 | 6.04 | 1 | 54.5 | 1 |
| *Stenopterobia intermedia* (Lewis) Fricke | Si | 35 | 5.71 | 5 | 23.1 | 7 |
| *Surirella delicatissima* Lewis | Sd | 39 | 5.50 | 7 | 13.8 | 9 |

[a] PIRLA, Paleolimnological Investigation of Recent Lake Acidification, project taxonomy (Camburn et al. 1984–86).

TABLE 5. Pearson (PN) and Spearman (SN) correlations of DECORANA axes with environmental variables for the full (63-lake) and culled (56-lake) data sets. $^{**}p = 0.001$; $^{*}p = 0.05$.

| | $N = 63$ | | | | $N = 56$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Axis 1 | | Axis 2 | | Axis 1 | | Axis 2 | |
| Variable | PN | SN | PN | SN | PN | SN | PN | SN |
| pH | 0.95** | 0.95** | 0.09 | 0.02 | 0.92** | 0.93** | 0.19 | 0.05 |
| Alk | 0.63** | 0.94** | 0.26* | 0.06 | 0.87** | 0.91** | 0.20 | 0.01 |
| Cond | 0.58** | 0.62** | 0.22 | 0.12 | 0.46** | 0.46** | 0.21 | −0.07 |
| Na | 0.14 | 0.34* | −0.05 | −0.12 | 0.17 | 0.36* | 0.12 | 0.09 |
| K | 0.18 | 0.34* | 0.07 | −0.05 | 0.19 | 0.36* | 0.36* | 0.05 |
| Mg | 0.61** | 0.81** | 0.15 | 0.05 | 0.71** | 0.73** | 0.03 | −0.07 |
| Ca | 0.60** | 0.82** | 0.23 | 0.20 | 0.71** | 0.75** | 0.26 | −0.14 |
| F | −0.14 | 0.23 | 0.20 | 0.44** | −0.13 | 0.18 | 0.34* | −0.13 |
| Cl | 0.02 | 0.11 | −0.20 | −0.24 | 0.07 | 0.15 | 0.18 | 0.17 |
| Mar-$SO_4$ | 0.02 | 0.11 | −0.21 | −0.23 | 0.07 | 0.15 | 0.18 | 0.17 |
| Non-mar-$SO_4$ | 0.24 | 0.10 | 0.30* | 0.49** | −0.09 | −0.07 | 0.00 | −0.22 |
| $NO_3$ | 0.04 | 0.12 | 0.39* | 0.48** | −0.13 | 0.00 | 0.28* | −0.23 |
| $NH_4$ | −0.12 | −0.24 | 0.23 | 0.22 | −0.13 | −0.40* | −0.08 | −0.17 |
| Total Al | −0.60** | −0.75** | 0.24 | 0.28* | −0.56** | −0.67** | 0.16 | −0.13 |
| Exch Al | −0.56** | −0.82** | 0.19 | 0.27* | −0.55** | −0.78** | 0.24 | −0.09 |
| Total P | 0.13 | 0.12 | 0.54** | 0.57** | −0.12 | −0.09 | 0.01 | −0.30* |
| $SiO_2$ | 0.35* | 0.48** | 0.31* | 0.27* | 0.24 | 0.41* | −0.03 | −0.04 |
| Chl *a* | 0.17 | 0.42** | 0.06 | 0.00 | 0.09 | 0.34* | −0.08 | −0.06 |
| DOC | 0.12 | 0.14 | 0.05 | 0.14 | 0.15 | 0.17 | −0.16 | −0.15 |
| Color | 0.03 | 0.18 | 0.35* | 0.38* | 0.01 | 0.13 | −0.10 | −0.22 |
| Area | 0.30* | 0.46** | −0.36* | −0.52** | 0.44** | 0.65** | 0.71** | 0.17 |
| Max. depth | 0.29* | 0.32* | −0.30* | −0.34* | 0.40* | 0.40* | 0.10 | 0.18 |
| Rel. depth | −0.20 | −0.14 | 0.26* | 0.27* | −0.31* | −0.31* | 0.05 | −0.07 |
| Transparency | 0.02 | 0.01 | −0.41** | −0.45** | 0.02 | 0.13 | 0.18 | 0.33* |
| Litt | −0.39* | −0.41* | −0.01 | 0.02 | −0.40* | −0.43* | 0.04 | 0.08 |
| Watershed area | 0.34* | 0.44** | −0.34* | −0.22 | 0.50** | 0.65** | 0.63** | 0.02 |
| *W/L* | −0.24 | −0.16 | 0.31* | 0.58** | −0.24 | −0.22 | 0.27* | −0.37* |
| Alt. | −0.42** | −0.38* | 0.19 | 0.27* | −0.39* | −0.36* | −0.08 | −0.06 |
| Lat. | 0.31* | 0.23 | −0.17 | −0.29* | 0.31* | 0.25 | −0.08 | 0.24 |

Granite (Billings 1956) which contains up to 1% F in biotite ($K(Fe,Mg)_3AlSi_3O_{10}(OH,F)_2$) and topaz ($Al_2SiO_4[OH,F]$) or fluorite ($CaF_2$) (Canney et al. 1987). Component 4 (10.7%var), with heavy positive loadings for water color and DOC, indicates colored organic solutes despite the short gradients of these variables in the data set. The negative loading for litt is largely a result of reduced transparency. This component also indicates trophic state (positive loadings for chlorophyll *a* and total P). Component 5 (9.3%var) indicates lake depth and surface area. Positive loadings for those variables are associated with small proportion of littoral area and small *W/A* ratio.

The ordination of the 63 sample (lake) scores on the first two PC axes (Fig. 3) indicates low scores on axis 1 for the 12 reconstruction lakes, consistent with their low concentrations of base cations, low pH (≤6.1), and very low alk (≤21 $\mu eq \cdot L^{-1}$) ( Table 1). Seven lakes, five in Vermont (lakes 53, 56, 59, 62, and 63) and two in Maine (lakes 8 and 32), form a loose separate group at the opposite (positive) end of axis 1, due to their relatively high concentrations of base cations and relatively high pH (7.1–7.7) and alk (≥386 $\mu eq \cdot L^{-1}$) (Table 1). On axis 2, the values are spread broadly, reflecting wide ranges of altitude and marine aerosols.

## Relationships of Diatoms to Environmental Gradients

The 113 taxa mentioned in this and related papers (Norton et al. 1985; Davis et al. 1990, 1994; Anderson et al. 1993) are listed in Table 4. Anderson et al. (1993) compared the values of autecological parameters (e.g., AWM pH) of the important taxa in our data set with those of data sets from other regions.

### *DCA, and indirect gradient analysis*

The variance in the diatom data is concentrated on DCA axis 1 (eigenvalue 0.49). Correlations of the axis with the 29 environmental variables indicate that it is positively related to pH, alk, Ca, Mg, and cond and negatively to total and exch Al (Table 5). The lower Pearson correlations for alk, Ca, and Mg reflect the nonlinear distributions of these variables. The 12 reconstruction lakes have diatom counts at the low end of the axis (Fig. 4). At the high end are counts from lakes with relatively high values of pH, alk, and related variables (low Al) and chlorophyll *a* and surface area. The seven lakes that scored highest on PCA axis 1 (Fig. 3) are near the high end of DCA axis 1.

Diatom taxa with highest scores on DCA axis 1 and characteristic of the relatively high-pH and high-alk lakes include *Fragilaria crotonensis*, *Cyclotella michiganiana*, *Achnanthes didyma*, *Asterionella formosa*, and *Cocconeis* spp. Taxa with strongly negative scores on the axis and characteristic of low-pH, low-alk lakes include *Tabellaria binalis* and *T. quadriseptata*, *Eunotia bidentula*, *Neidium iridis*, and *N. bisulcatum* (Table 6).

DCA axis 2 (eigenvalue 0.12) is statistically much weaker than axis 1 and is positively correlated with total P, $NO_3$, *W/L*, color, and non-mar-$SO_4$ and negatively with lake

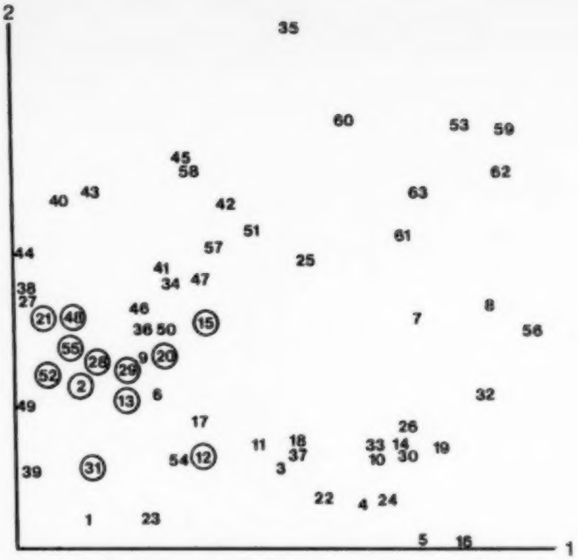

FIG. 4. Ordination of the 63 lake diatom counts on DCA axes 1 and 2, based on 187 taxa. Names and characteristics of numbered lakes are given in Table 1. Circled numbers indicate lakes for which paleolimnological reconstructions have been carried out (Davis et al. 1983, 1990, 1994; Norton et al. 1985).

surface area and transparency (Table 5). The lower Pearson correlations for F, non-mar-$SO_4$, area, and *W/L* reflect the nonlinear distributions of these variables.

### *Diatom clusters*

The cluster membership of each taxon is given in Table 4. Taxa exemplifying each cluster are plotted on the pH and alk gradients in Fig. 5. The plots show all the variance in the raw data, although cluster definition itself was based on slightly smoothed data. The plots indicate strong relationships between taxa and pH and alk, but the distributions are noisy because additional factors affect the diatom abundances. Of the seven pH clusters, cluster 1 represents the high end and cluster 7 the low end of the gradient. Had there been a more extended pH scale (e.g., 3–12), the taxa in these two clusters probably would have exhibited clear unimodal distributions. However, on our pH 4.45–7.91 gradient, the distributions of most of the common taxa in these clusters are so truncated as to be essentially uniclinal. For cluster 1 (all taxa), an *F*-test indicated no significant improvement of a second- over a first-degree regression; for cluster 7, there was a significant but small improvement ($r^2$ 0.777 versus 0.705). One-hundred and twenty of the 187 taxa belong to these two clusters (Table 4). The remaining clusters are more clearly unimodal, with the mode ranging from high (cluster 2) to low pH (cluster 6), and with some clusters more skewed than others.

Distinctions between the middle clusters on the basis of mode and overall curve shape are unclear, as between *Melosira distans* var. *tenella* in cluster 3, *M. distans* var. *nivaloides* in cluster 4, and *Fragilaria virescens* var *exigua* in cluster 6 on the pH axis. A separate cluster analysis of the 63 complete diatom assemblages formed clusters from the numerous lakes in the mid-pH range. Each of these midrange clusters formed a group of lakes that differed from the others as much in morphometry as in pH and alk (Anderson et al. 1986). Apparently, in the taxa clustering, much of this morphometry-related variance survived the two-point running means.

TABLE 6. Twenty highest and lowest scores for taxa occurring in at least 10 lakes on DCA axis 1 for the full (63-lake) and culled (56-lake) calibration data sets. In parentheses, the number of lakes of occurrence is given after each taxon, and the abundance-weighted mean pH is given for taxa not in Table 4.

| Taxon | Scores | |
|---|---|---|
| | $N = 63$ | $N = 56$ |
| *Fragilaria crotonensis* (11) | 486 | |
| *Cyclotella michiganiana* (15) | 466 | 426 |
| *Achnanthes didyma* (11) | 397 | 410 |
| *Asterionella formosa* (23) | 388 | 394 |
| All *Cocconeis* spp. (10) | 380 | 357 |
| *Navicula radiosa* (13) | 380 | 367 |
| *Fragilaria brevistriata* (14) | 375 | |
| *Fragilaria construens* (21) | 372 | |
| *Cymbella microcephala* (14) | 365 | |
| *Navicula* sp. 4 PIRLA (11) | 358 | 336 |
| *Fragilaria vaucheriae* (11) (7.14) | 357 | |
| *Achnanthes linearis* f. *curta* (11) | 355 | 347 |
| *Cyclotella comta* (26) | 351 | 362 |
| Other *Cyclotella* spp. (11) | 344 | |
| *Fragilaria pinnata* (32) | 344 | 321 |
| *Synedra rumpens* (17) | 340 | 322 |
| *Navicula pseudoscutiformis* (12) | 335 | 329 |
| *Melosira ambigua* (24) | 330 | 345 |
| *Achnanthes minutissima* (31) | 324 | |
| *Melosira italica* ssp. *subarctica* (16) | 310 | 361 |
| *Synedra filiformis* var. *exilis* (16) | | 356 |
| *Tabellaria flocculosa* str. IIIp (21) | | 324 |
| *Achnanthes microcephala* (18) (6.37) | | 324 |
| *Cyclotella stelligera* (37) | | 322 |
| *Achnanthes linearis* (19) | | 310 |
| *Navicula cocconeiformis* (14) (6.51) | | 304 |
| Other *Synedra* spp. (29) | | 303 |
| *Eunotia pectinalis* var. *minor* (42) | | −10 |
| *Pinnularia abaujensis* var. 2 PIRLA (25) | | −13 |
| *Frustulia rhomboides* var. *saxonica* (44) | 4 | |
| *Fragilaria oldenbergiana* (10) (5.30) | 0 | |
| *Eunotia tenella* (34) | −1 | 7 |
| *Semiorbis hemicyclus* (23) | −10 | −1 |
| *Eunotia serra* (15) | −20 | −17 |
| *Navicula subtilissima* (38) | −21 | −13 |
| *Eunotia microcephala* (12) (5.02) | −31 | −25 |
| *Navicula tenuicephala* (12) | −33 | −26 |
| *Neidium iridis* var. *amphigomphus* (25) | −37 | −32 |
| *Frustulia magaliesmontana* (30) | −41 | −34 |
| *Eunotia sudetica* (10) (5.02) | −44 | −39 |
| *Neidium affine* (36) | −52 | −49 |
| *Pinnularia microstauron* (21) | −67 | −66 |
| *Neidium* sp. 2 PIRLA (10) | −74 | −76 |
| *Actinella punctata* (23) | −82 | −78 |
| *Neidium bisulcatum* (17) | −86 | −85 |
| *Tabellaria quadriseptata* (28) | −87 | −107 |
| *Neidium iridis* (15) | −89 | −83 |
| *Eunotia bidentula* (15) | −107 | −106 |
| *Tabellaria binalis* (10) | −168 | −167 |

### *Canonical correspondence analysis (CCA)*

The percentage of the species–environment variance accounted for by each of the first four CCA axes (and their

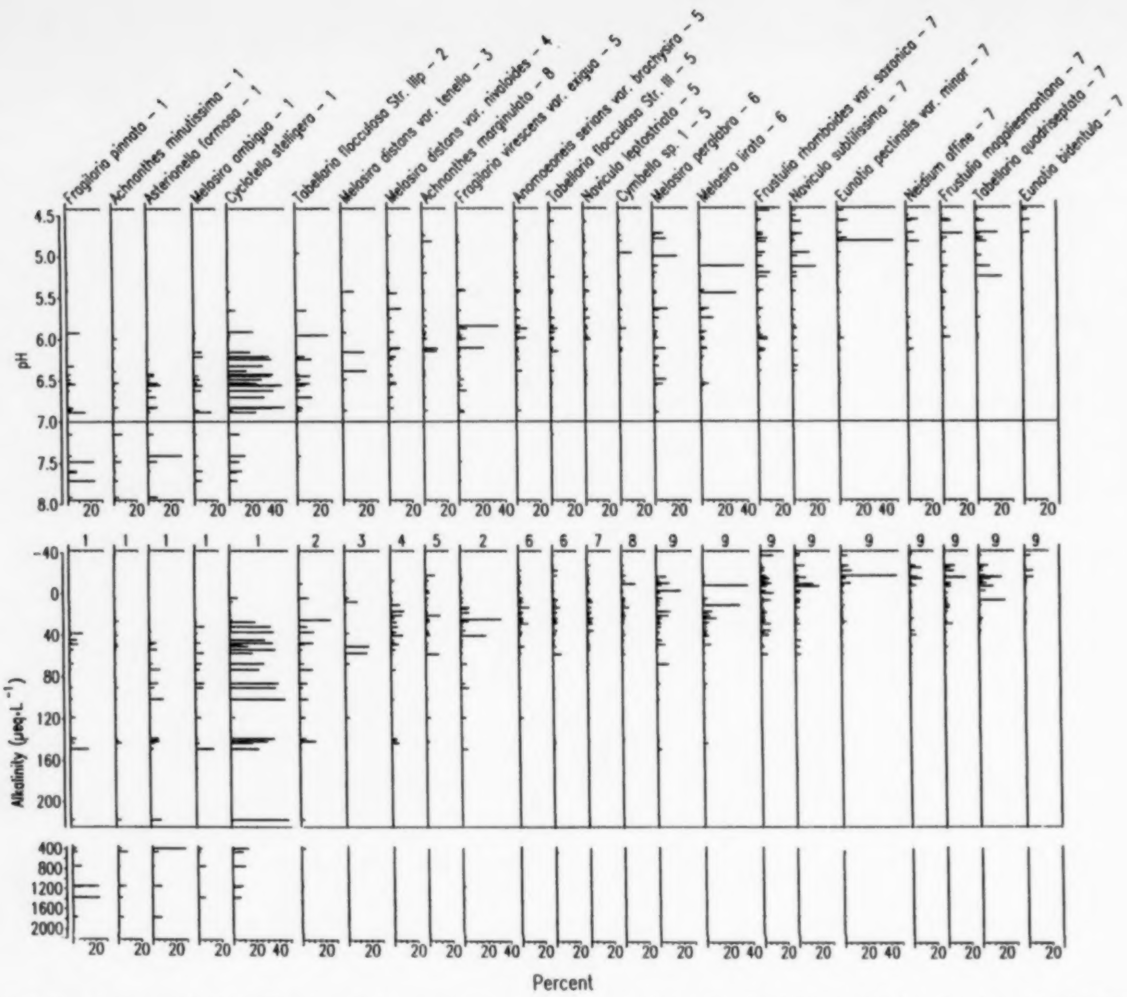

FIG. 5. Taxa important in the calibrations and reconstructions, and characteristic of each of the clusters (numbers) used in the CLUS regressions (Tables 4 and 9), plotted on pH and alk scales. On each scale, the 56-lake data set is above the horizontal line. Note change in scale of alk.

eigenvalues) are (1) 17.3 (0.66), (2) 13.8 (0.53), (3) 10.8 (0.41), and (4) 6.9 (0.27). These percentages and the total of them are substantial for field data made up of so many biological and environmental variables (ter Braak 1987). The intraset correlations (IC) demonstrate the relative contributions of the environmental variables to the CCA axes (Table 7). The canonical coefficients are not given because they are unstable due to strong correlations among environmental variables (e.g., pH with several other variables) (ter Braak 1986). Most highly ($p = 0.001$) correlated with axis 1 are pH, alk, Mg, Ca, and Cond (*positive*) and total Al, exch Al, altitude, and littoral index (*negative*). This result agrees with the prior ones that the dominant environmental gradient is a combination of pH and related variables.

Correlations with CCA axis 2 are much weaker and largely positive, viz. $SiO_2$, water color, total Al, F, and *W/L* (Table 7). Except for its stronger score for water color, and weak scores for $NO_3$ and K, the axis roughly approximates PCA axis 3 (Table 3).

In a second CCA, the effect of $\log_{10}$-transformation of alk is seen in improved IC for alk, from 0.66 to 0.86 (axis 1) and from 0.15 to 0.26 (axis 2), but also in worsened ICs for the large majority of the other variables, by 0.01 or 0.02 for most and by 0.04 for a few. Apparently, these gains and losses canceled each other in their effects on summary statistics: the percentage of the species–environment variance accounted for by each of these first four CCA axes (and their eigenvalues) are (1) 16.8 (0.66), (2) 12.6 (0.50), (3) 11.6 (0.46), and (4) 9.6 (0.38), very similar to the figures given for the CCA without transformation of alk.

The environmental vectors on the biplots (Fig. 6) reflect the intraset correlations (Table 7). The 63 diatom sample scores are plotted (Fig. 6a), as are the scores for the 63 taxa (Fig. 6b) that are most abundant in the samples and of importance in the 12 reconstructions (Norton et al. 1985; Davis et al. 1990, 1994). The diatom sample and taxa scores are well spread on axis 1, but there is little spread on axis 2 except for a small number of cases near the acidic end of axis 1. Thus the CCA directly shows what was indirectly shown in the previous analyses: that the dominant pattern in the diatom data is most closely related to a complex of pH and related variables. This strong relationship indicates that these diatom counts are likely to serve well for deriving transfer coefficients for inferential models of pH and related variables.

Diatom taxa with high scores on CCA axis 1 (Fig. 6b) and associated with relatively high pH and alk are *Fragilaria crotonensis*, *F. vaucheriae*, *F. construens*, *Cyclotella michiganiana*, and *Cymbella microcephala*. These taxa are rare or absent in the reconstruction lake cores (Norton et al. 1985; Davis et al. 1990, 1994). Taxa with low scores and associated with low pH and alk are *Tabellaria binalis* and *T. quadriseptata*, *Eunotia bidentula*, *E. pectinalis* var. *minor*, and *Pinnularia microstauron*. These taxa are common in the reconstruction lakes cores.

The four diatom samples that are farthest from the reconstruction lakes on axis 2 are from the acidic New Hampshire lakes Ethan (40), Cone (38), Darrah (39), and Unknown (49). Ethan and Cone ponds have exceptionally high total and exch Al. Ethan has very high F, SiO$_2$, color, and total P and near-median sum of base cations (Sum-BC) and non-mar-SO$_4$. Cone has near-median F, SiO$_2$, and total P, very low color and Sum-BC, and high non-mar-SO$_4$ (Table 1). *Fragilaria acidobiontica*, which occurred in only six of the lakes (Table 4), was present at 81.1 and 14.2%, respectively, in Ethan and Cone ponds. The other four sites containing this species (lakes 20, 41, 44, and 55 in Table 1 and Fig. 1), at lower percentages, all have about the same as or greater than median total and exch Al. These six lakes are located in the southwestern half of the study region, toward the Adirondacks, N.Y., where Charles (1986), Charles et al. (1990), and Dixit et al. (1993) suggested that the species is a good indicator of high concentrations of total and "monomeric" Al. Our study supports that suggestion. Darrah and Unknown ponds have relatively low Al for their very low pH, very low Sum-BC, and exceptionally low SiO$_2$ (Table 1). These lakes have 54.2 and 34.8% *Asterionella ralfsii* var. *americana* (short form).

### *Conclusions regarding the 63-lake data set*

These analyses indicate that the strongest environmental relationship of the diatoms is with the group of chemical variables pH, alk, Ca, and Mg. With a small number of exceptions, taxa distributions are only weakly related to the other environmental factors. Thus we conclude that the 63-lake data set is well suited for calibration of regression models for pH and alk. However, inferential models calibrated with this data set are more appropriate for some reconstruction lakes than others.

## Culled Data Set (56 Lakes)

PCA and DCA revealed a group of seven lakes that is widely divergent from our 12 reconstruction lakes along the acidity and diatom gradients. This group consists of West Garland (32), Goodall (53), Hough (56), North King (59), Walton (62), and Westford (63) ponds and Hunnewell Lake (8) (Table 1). In a cluster analysis of the 63 diatom assemblages, the group-of-seven formed a discrete cluster (Anderson et al. 1986). In a DCA to which the 36 down-core assemblages from Haystack, Mud, and Little Long ponds (Table 1) were added to the 63 surface assemblages, the cores and the group-of-seven were at opposite ends of axis 1.

TABLE 7. Intraset correlations of CCA axes with environmental variables for the 63-lake and 56-lake calibration data sets. $^{**}p = 0.001$; $^{*}p = 0.05$.

| Variable | *N* = 63 |  | *N* = 56 |  |
|---|---|---|---|---|
|  | Axis 1 | Axis 2 | Axis 1 | Axis 2 |
| pH | 0.95** | 0.16 | −0.88** | 0.30* |
| Alk | 0.66** | 0.15 | −0.82** | 0.31 |
| Cond | 0.62** | 0.13 | −0.44** | 0.05 |
| Na | 0.14 | 0.03 | −0.16 | 0.04 |
| K | 0.18 | 0.05 | −0.18 | 0.08 |
| Mg | 0.66** | 0.16 | −0.67** | 0.18 |
| Ca | 0.65** | 0.17 | −0.64** | 0.32* |
| F | −0.19 | 0.38* | 0.23 | 0.34* |
| Cl | 0.04 | −0.08 | −0.10 | −0.07 |
| Mar-SO$_4$ | 0.04 | −0.08 | −0.10 | −0.07 |
| Non-mar-SO$_4$ | 0.31* | 0.20 | 0.11 | 0.13 |
| NO$_3$ | 0.01 | 0.13 | 0.16 | 0.10 |
| Total Al | −0.65** | 0.40** | 0.67** | 0.33* |
| Exch Al | −0.58** | 0.23 | 0.59** | 0.15 |
| Total P | 0.07 | 0.23 | 0.19 | 0.23 |
| SiO$_2$ | 0.32* | 0.47** | −0.13 | 0.49** |
| Chl *a* | 0.20 | −0.11 | −0.13 | −0.12 |
| DOC | 0.08 | 0.17 | −0.10 | 0.20 |
| Color | −0.04 | 0.45** | 0.12 | 0.44** |
| Area | 0.29* | 0.01 | −0.43** | 0.11 |
| Max. depth | 0.36* | −0.12 | −0.49** | −0.02 |
| Rel. depth | −0.15 | −0.18 | 0.23 | −0.24 |
| Litt | −0.45** | 0.15 | 0.48** | 0.07 |
| *W/L* | −0.28* | 0.25* | 0.32* | 0.20 |
| Alt. | −0.49** | 0.15 | 0.48** | 0.08 |
| Lat. | 0.31* | 0.08 | −0.32* | 0.16 |

Four of the seven lakes (32, 53, 56, and 59) had residuals exceeding 1.75 RMSE in different alk model regressions with and without log-transformation of alk, another (8) in only one alk regression, and two (53 and 56) in pH regressions. All had pH and alk residuals >1.0 RMSE. Effects of this group of lakes on distributions of residuals over specific parts of the gradients, crucial to the culling decision, are described later. All told, these results argue for the culling of these lakes from the data set to be used for calibration of models for application at our acidic reconstruction lakes.

The resultant 56-lake data set was subjected to the same statistical analyses as had been performed on the 63-lake set to confirm its suitability as a calibration set. The primary PCA and DCA axes and their interpretations remain virtually the same (Tables 3, 5, and 6). However, unlike the 63-lake PCA, lake morphometric variables, viz. lake area, maximum and relative depth, and *W/L*, have heavy or moderate loadings on axis 1. The percentage of the total variance accounted for by each of the first four CCA axes (and their eigenvalues) are (1) 17.6 (0.61), (2) 15.4 (0.54), (3) 8.5 (0.30), and (4) 6.5 (0.22). The relationships among environmental gradients (Table 7), diatom counts, and diatom taxa (Table 8) on the first two CCA axes are very similar to those of the 63-lake CCA. However, in contrast with the 63-lake analysis, the effect of transformation of alk was to reduce its IC on axis 1 (from −0.82 to −0.74). The percentage of the total variance accounted for by each of the first four CCA axes (and their eigenvalues), viz. (1) 16.1 (0.61), (2) 14.5 (0.53), (3) 12.2 (0.44), and (4) 66.1 (0.22), are very

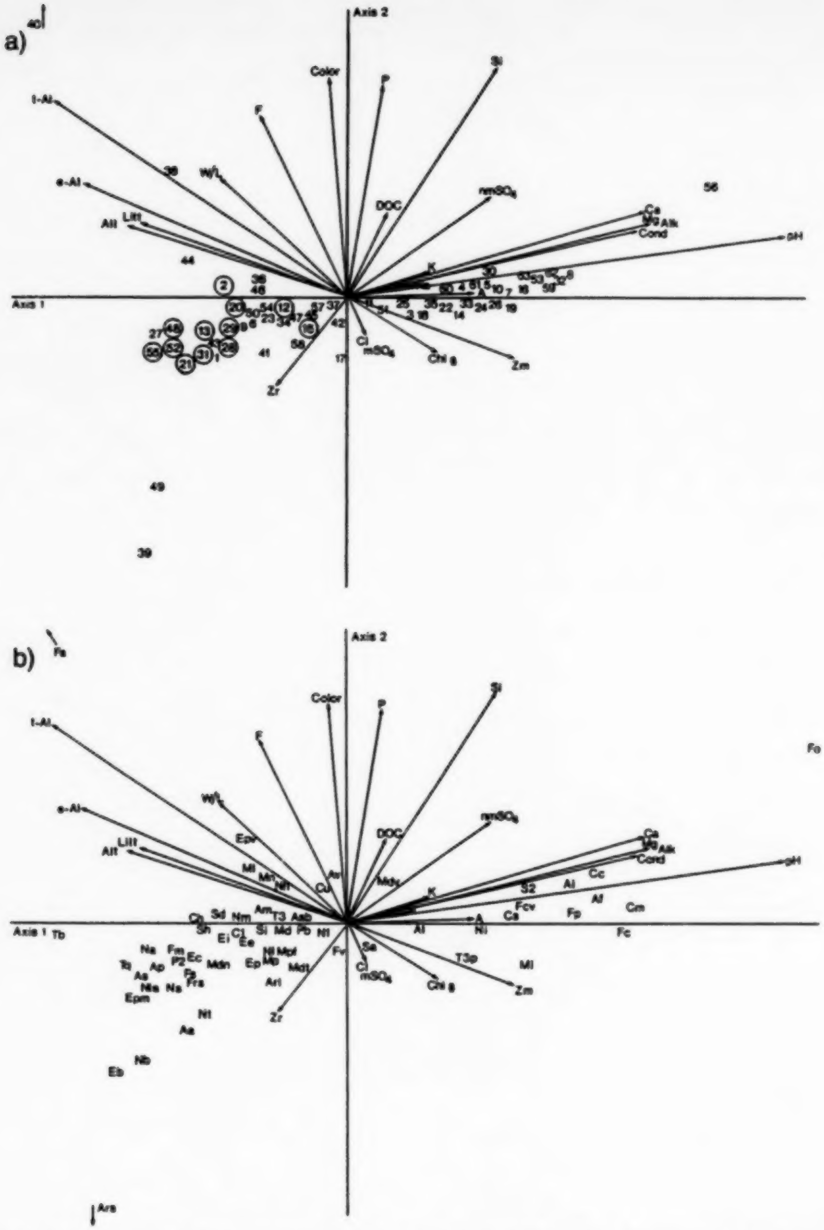

FIG. 6. (a) Biplot on CCA axes 1 and 2 showing environmental vectors and 63 lake sample scores. Based on data without transformation. Names and characteristics of numbered lakes are given in Table 1. (b) Biplot on CCA axes 1 and 2 showing environmental vectors and species scores. Species codes are defined in Table 4.

similar to those just given for the 56-lake CCA without transformation of alk. We conclude that in the 56-lake data set the diatom/pH and diatom/alk relationships are about as strong as in the 63-lake set.

## Results, Part 2. Inferential Models and Calibrations for pH and Alkalinity

Three inferential models, DCA, CLUS, and CCA, were calibrated for six data options, yielding 18 calibrated regressions. For pH, the three models were calibrated using 63- and 56-lake data sets and for alk using these two data sets with and without $\log_{10}$-transformation of alk.

### pH

The $r^2$ is a little higher for the 63-lake (0.87–0.91) than 56-lake (0.84–0.86) regressions, and the RMSE is very similar (smaller by 0.06 unit for 56-lake CLUS). The three models have very similar summary statistics, with CCA slightly better overall (Table 9). For CLUS and CCA, the 56-lake regressions have mean residuals about equal to their 63-lake counterparts for lakes with predicted $\mathrm{pH} < 6.5$. Residuals for that pH range for DCA average ~0.02 unit higher for the 56-lake data set (Fig. 7).

### Untransformed Alkalinity

The DCA and CLUS regressions based on 63 lakes are very sensitive to the skewed alk distribution, yielding $r^2$ of only 0.40, and high RMSE (272 and 290 μeq·L$^{-1}$). The CCA regression is much better ($r^2 = 0.84$; RMSE = 150), reflecting the capacity of CCA to maximize the fit to its axes of the combined biological and environmental data, but retains some skew (note change in scale in Fig. 7). The culling of the seven highest alk lakes, by removing most of the skew (Fig. 2), considerably improves the $r^2$ for DCA and CLUS (to 0.75 and 0.79), and greatly reduces the RMSE to 27 μeq·L$^{-1}$. The 56-lake CCA statistics barely differ from those of DCA and CLUS (Table 9). The three 56-lake regressions have residuals that average 57 μeq·L$^{-1}$ smaller for lakes with <100 μeq predicted alk, and 107 μeq·L$^{-1}$ smaller for lakes with 100–300 μeq predicted alk, than their 63-lake counterparts (Fig. 7).

### Log-transformed Alkalinity

For the 63-lake set, transformation of alk substantially improves summary statistics for the DCA and CLUS regressions, but worsens them for the CCA regression (Table 9). However, summary statistics give an incomplete picture. Although transformation reduces residuals by an average of 49 μeq·L$^{-1}$ for predicted alks of 100–300 μeq·L$^{-1}$ and by 71 μeq·L$^{-1}$ for predicted negative alks, the reduction averages only 17 μeq·L$^{-1}$ for the numerous predicted alks of 0–100 μeq·L$^{-1}$, and residuals are enlarged by 174–348 (mean 243 μeq·L$^{-1}$) for predicted alks over 300 μeq·L$^{-1}$ (Fig. 7). Parallel comparisons of summary statistics and residuals for the 56-lake regressions yield more consistent results: transformation makes little difference because without the seven highest alkalinity lakes the distribution of alkalinity is near-linear (Fig. 2). The largest difference is for predicted negative alks, where transformation enlarges mean residuals for DCA and CLUS (6 and 4 μeq·L$^{-1}$, respectively) and reduces them for CCA (8 μeq·L$^{-1}$). Overall, the best regressions for alk inference at our acidic reconstruction lakes are the 56-lake untransformed ones.

## Discussion

### Outliers

For the 56-lake regressions (pH and untransformed alk), we designate as outliers those samples with residuals that exceed 0.50 pH unit or 50 μeq·L$^{-1}$ alk (3–9%). Study of outliers reveals some of the circumstances that may lead to incongruous diatom assemblages and inaccurate inferences. Jackson Pond (No. 9) with measured pH 4.75 has a combined abundance (~25%) of *Melosira* species characteristic of higher pH lakes. All three models infer a pH that is too high (Fig. 7). One possible cause for this incongruity is sediment mixing. Field notes indicate that a small amount of degassing (rising $CH_4$ (?) bubbles) occurred in the highly organic and flocculent sediment of the core, probably introducing old diatom remains to the surface sediment. If Jackson Pond had acidified in recent years, the contaminants (rich in *Melosira*?) would cause an erroneously high pH inference. Natural in situ degassing and bioturbation can have similar effects. More substantial degassing in cores from two other lakes led to their omission from the study. In our experience, degassing caused by temperature increase and/or pressure reduction upon raising a core is most likely to occur when sediments are highly organic and flocculent.

TABLE 8. Twenty highest and lowest scores for taxa occurring in at least 10 lakes on CCA axis 1 for the 63-lake and 56-lake calibration data sets. Note that the environmentally equivalent ends of the axis have reverse signs. In parentheses, the number of lakes of occurrence is given after each taxon, and the abundance-weighted mean pH is given for taxa not in Table 4.

| Taxon | Scores |  |
|---|---|---|
|  | $N = 63$ | $N = 56$ |
| *Fragilaria crotonensis* (11) | 483 | −205 |
| *Cyclotella michiganiana* (15) | 299 | −310 |
| *Fragilaria vaucheriae* (11) (7.14) | 290 |  |
| *Fragilaria construens* (21) | 285 | −202 |
| *Cymbella microcephala* (14) | 262 |  |
| *Asterionella formosa* (23) | 261 | −277 |
| *Cyclotella comta* (26) | 255 | −252 |
| *Achnanthes minutissima* (31) | 235 | −189 |
| *Fragilaria pinnata* (32) | 234 | −203 |
| All *Cocconeis* spp. (10) | 232 | −217 |
| *Synedra rumpens* (17) | 214 | −194 |
| *Achnanthes didyma* (11) | 211 | −270 |
| *Navicula radiosa* (13) | 211 | −197 |
| *Achnanthes linearis* f. *curta* (11) | 202 | −239 |
| *Melosira ambigua* (24) | 194 | −241 |
| *Fragilaria brevistriata* (14) | 194 |  |
| *Melosira italica* ssp. *subarctica* (16) | 190 | −253 |
| *Synedra* sp. 2 PIRLA (18) | 190 |  |
| Other *Cyclotella* spp. (11) | 188 | −209 |
| Other *Synedra* spp. (29) | 185 |  |
| *Cyclotella stelligera* (37) |  | −235 |
| *Synedra filiformis* var. *exilis* (16) |  | −225 |
| *Navicula* sp. 4 PIRLA (11) |  | −200 |
| *Tabellaria flocculosa* str. IIIp (21) |  | −190 |
| *Navicula pseudoscutiformis* (12) |  | −181 |
| *Eunotia curvata* (44) |  | 126 |
| *Fragilaria constricta* (26) | −159 |  |
| *Eunotia vanheurckii* var. *intermedia* (29) | −159 | 130 |
| *Achnanthes austriaca* var. *helvetica* (10) | −162 | 137 |
| Other *Eunotia* spp. (51) | −162 | 123 |
| *Eunotia sudetica* (10) (5.02) | −163 | 130 |
| *Pinnularia abaujensis* var. 2 PIRLA (25) | −171 | 144 |
| *Navicula subtilissima* (38) | −173 | 139 |
| *Frustulia magaliesmontana* (30) | −176 | 145 |
| *Neidium iridis* (15) | −187 | 144 |
| *Actinella punctata* (23) | −189 | 151 |
| *Neidium iridis* var. *amphigomphus* (25) | −199 | 150 |
| *Neidium affine* (36) | −203 | 165 |
| *Neidium* sp. 2 PIRLA (10) | −205 | 187 |
| *Eunotia microcephala* (12) (5.02) | −209 | 172 |
| *Neidium bisulcatum* (17) | −209 | 172 |
| *Pinnularia microstauron* (21) | −210 | 187 |
| *Eunotia pectinalis* var. *minor* (42) | −219 | 196 |
| *Tabellaria quadriseptata* (28) | −220 | 189 |
| *Eunotia bidentula* (15) | −239 | 196 |
| *Tabellaria binalis* (10) | −298 | 269 |

A second possible cause of an incongruous assemblage in a lake whose chemistry has recently changed is time lag in

TABLE 9. Summary statistics for 18 regressions, and the six 56-lake regressions used by Davis et al. (1990, 1994). RMSE = root mean square error. Eigenvalues for DCA axis 1 are based on only the biological data. For comparability with the other regressions, $r^2$ and RMSE of regressions of log-transformed alk are based on the relationships between nonlog measured values and antilogs of the predicted log values. For these regressions, the $r^2$ and RMSE based directly on the logs are given in parentheses. The probabilities in $F$-tests that the null hypotheses are true are <0.0001 in all but the 63-lake CLUSTER regression for untransformed alk for which $p$ = 0.0007 (see text regarding the problematic nature of these probabilities). See Materials and Methods for generalized CCA regression equation.

| | $N$ = 63 | | | $N$ = 56 | | |
|---|---|---|---|---|---|---|
| Regression | $r^2$ | RMSE | Eigenvalue | $r^2$ | RMSE | Eigenvalue |
| pH | | | | | | |
| DCA | 0.90 | 0.29 | 0.49 | 0.84 | 0.29 | 0.42 |
| CLUSTER | 0.87 | 0.35 | | 0.86 | 0.29 | |
| CCA | 0.91 | 0.28 | 0.62 | 0.86 | 0.29 | 0.54 |
| Alk | | | | | | |
| DCA | 0.40 | 272 | 0.49 | 0.75 | 27 | 0.42 |
| Log | 0.68 | 241 | 0.49 | 0.76 | 29 | 0.42 |
| | (0.74) | (0.29) | | (0.61) | (0.27) | |
| CLUSTER | 0.40 | 290 | | 0.79 | 27 | |
| Log | 0.54 | 272 | | 0.78 | 25 | |
| | (0.76) | (0.30) | | (0.74) | (0.24) | |
| CCA | 0.84 | 150 | 0.48 | 0.77 | 29 | 0.48 |
| Log | 0.77 | 190 | 0.60 | 0.78 | 29 | 0.54 |
| | (0.91) | (0.17) | | (0.90) | (0.14) | |

56-lake regressions

pH

DCA

$$\text{pH} = 4.7305 + 0.0076(x)$$

$x$ = diatom sample score on DCA axis 1

CLUSTER

$$\text{pH} = 6.6074 + 0.0026(\text{C1}) - 0.0098(\text{C2}) - 0.0032(\text{C3}) - 0.0054(\text{C4}) - 0.0090(\text{C5}) - 0.0117(\text{C6}) - 0.0239(\text{C7})$$

C = total percent representation of diatom taxa in numbered pH cluster in a sample

Alk (untransformed)

CCA

$$\text{pH} = 5.746 - \frac{0.726(x)}{1.090}$$

$x$ = diatom sample score on CCA axis 1 constrained to pH

DCA

$$\text{Alk} = -35.2423 + 0.5147(x)$$

$x$ = diatom sample score on DCA axis 1

CLUSTER

$$\text{Alk} = 125.8215 + 0.0619(\text{C1}) - 1.6312(\text{C2}) - 1.1190(\text{C3}) - 1.1595(\text{C4}) - 0.3303(\text{C5}) - 1.1975(\text{C6}) - 0.0220(\text{C7}) - 2.1718(\text{C8}) - 1.7208(\text{C9})$$

C = total percent representation of diatom taxa in numbered alk cluster in a sample

CCA

$$\text{Alk} = 33.886 + \frac{52.475(x)}{0.955}$$

$x$ = diatom sample score on CCA axis 1 constrained to alk

focusing of littoral benthic diatoms to the profundal coring site. Mountain Pond (No. 20) (Fig. 7) had a strongly acidophilic diatom assemblage (*Frustulia* spp., *Eunotia* spp. and *Anomoeoneis serians* var. *brachysira*) uncharacteristic of the lake's relatively high pH of 6.0. Planktonic diatoms were scarce in the sample. Davis et al. (1994) have demonstrated that the pH of this lake had recently increased due to logging of the catchment and offered the hypothesis of a focusing time lag to explain the incongruous surface sediment assemblage.

A third possible cause of an incongruous assemblage is overrepresentation by epipelic diatoms living at the coring site, as might occur in shallow and/or transparent lakes lacking a profundal zone. Our methods do not distinguish epipelic living diatoms from sedimented nonliving diatoms. This

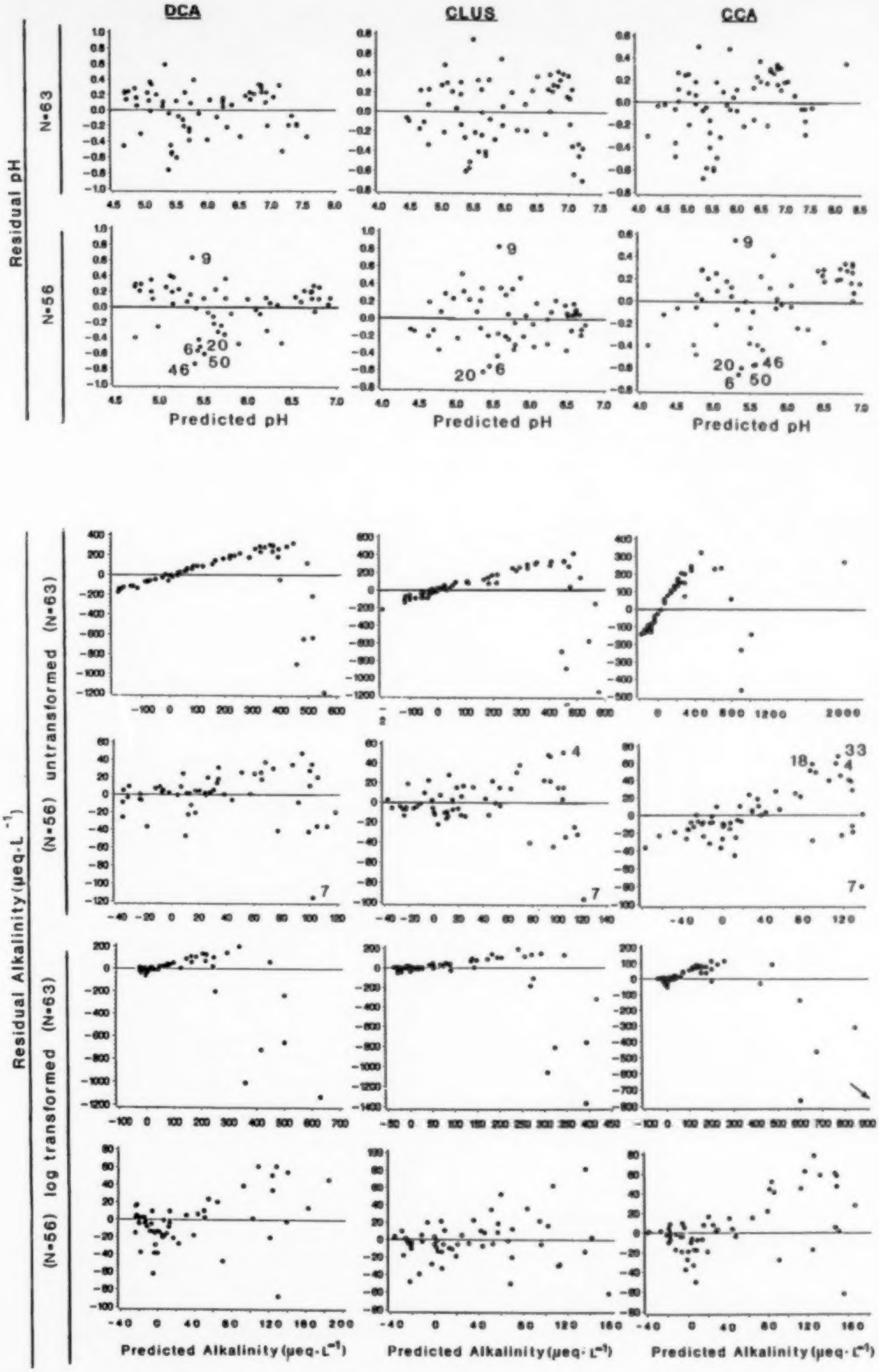

FIG. 7. Residual plots for 18 regressions. Outlier residuals (lake numbers as in Table 1) for the 56-lake regressions (pH and untransformed alk) are discussed in text.

explanation may apply to the outliers Upper and Lower Greeley ponds (Nos. 50 and 46 in Fig. 7), where transparency exceeded the greatest water depth (Table 1). This problem can be avoided by only coring lakes with a profundal zone, but this may not be possible, depending on the morphometric and other characteristics of lakes in a region.

A fourth possible cause is atypical lake morphology. Outliers with alk inferences that are too high, like Granger (No. 4), Beaver Mountain (No. 18), and Worthley (No. 33) ponds (Fig. 7) have assemblages with relatively high proportions of planktonic diatoms (with high abundance-weighted mean alk) for the lakes' relatively low measured alks of 54, 31, and 47 $\mu$eq·L$^{-1}$, respectively. These discrepancies may be due to the lakes' respective large areas of 51, 220, and 152 ha (data set mean 34 ha; median 6 ha) and the remoteness of their coring sites from shallow areas of abundant benthic diatom growth. Positive effects of lake area and depth on abundance of sedimentary remains of planktonic *Cyclotella* species have been noted by Taylor et al. (1987) for Canadian Shield lakes and by Anderson et al. (1993) for our lakes. The solution to this problem is to maintain uniform morphometry, similar to that of the reconstruction lakes, in data sets used for calibration of inferential models of water chemistry. In practice, this goal is only partly attainable, given the multiple chemical, physical, and other variables that must be considered in lake selection and the limited number of qualifying lakes in a region.

Despite the importance of lake morphometry to some taxon distributions, morphometry is unlikely to be important in the application of the models to the most recent 300 yr of lake sediment in this region. Only minor morphological changes would result from the small amount of sediment accumulation and slight lake level fluctuations in the ongoing moist climate of the period (Baron et al. 1980; Russell et al. 1993). Furthermore, diatom indicators of large lake area are virtually absent in cores from our 12 reconstruction lakes (Davis et al. 1990, 1994).

The incongruously high inferred alks of lakes 4, 18, and 33 may be partly due to the statistical effect on the regression of the most extreme outlier. Horseshoe Pond (No. 7) (Fig. 7), whose inferred alk is much too low. Its outlier position appears to result from its high measured alk (218 $\mu$eq·L$^{-1}$) relative to the rest of the 56-lake data set.

### Normality and *F*-tests

*F*-tests applied to regressions based on nonnormally distributed data yield invalid probabilities. Tests for normality of the data used in the 18 regressions failed for 15 and gave marginal results for the others (e.g., CCA 56-lake untransformed alk). For some of the 15, removal of one to three outliers results in much closer approximation of normality. However, the consistently extremely low probabilities, <0.0001 that $H_0$ is true, considered together with the other strong statistics for most of the regressions (Table 9), argue that the regressions are highly significant and that the violation of normality has a minimal effect.

### Biological Distributions and Model Assumptions

Biological data usually display unimodal distributions on environmental gradients. However, on the restricted gradients of our 63- and 56-lake data sets, most of the diatom taxa exhibit extremely truncated distributions. Sixty-four percent of the 113 taxa in Table 4 belong to pH clusters 1 and 7 or alkalinity clusters 1 and 9, which are practically uniclinal.

DCA assumes unimodal (Gaussian or bell-shaped) distributions of individual diatom taxa and computes WAs to characterize their modes on the derived axes. The diatom sample scores on axis 1 provide a good basis for simple linear regressions on pH and alk because of the high linear correlations (>0.9; $p = 0.001$) of the axis with those environmental variables (Table 5). As the DCA regressions are the only ones to use log-transformed diatom data, they yielded the most disparate results (Davis et al. 1990, 1994), producing relatively damped reconstructions, viz. change from sample to sample and total change through time were smaller than in reconstructions based on the other models. Damping was most severe when diatom assemblages were dominated by a very few taxa. Damping was further accentuated by the "shrinking effect" of the averaging employed by DCA (ter Braak and van Dam 1989); we did not include a deshrinking step in the DCA regressions. Cumming and Smol (1993) recommended that chryosphyte data in pH calibration data sets be transformed (log or square root) to minimize the effect of the dominant taxa which are often pH generalists and to emphasize the subdominant taxa which are more pH specialized. Strong dominance by diatom pH generalists generally does not appear to be a problem.

Each cluster is composed of a group of taxa with similar distributions on an environmental gradient. These "composite taxa" were used in a linear multiple-regression model regardless of their true distributions. In spite of that, the regressions have good statistics because of the strong effects of the large clusters that weigh heavily toward the ends of the axes. The model parallels the "selected taxa" linear multiple-regression model on pH of Davis and Anderson (1985), except by using groups rather than individual taxa, it partly overcomes the shortcoming of the earlier model that led to "…erratic pH inferences due to sensitivity to unusual frequencies of individual taxa". Nevertheless, inferences based on the CLUS regressions are more sensitive than the others to small shifts in diatom percentages, especially when a cluster with a high coefficient is involved (Davis et al. 1994). Although a cluster may contain many species, a down-core diatom assemblage may be dominated by only one or two species of the cluster.

As for the DCA model, the CCA model assumes bell-shaped distributions of diatom taxa and places them along environmental gradients using their WAs (ter Braak 1986). The strong intraset correlations for pH and alk on CCA axis 1 (>0.8 for $n = 56$; $p = 0.001$; Table 7) supported the use of simple linear regressions of the diatom sample scores on axis 1 constrained to pH and alk, respectively. CCA statistics are markedly superior to DCA statistics for the 63-lake regression of alk without transformation because CCA maximizes its axes in relation to both the environmental and biological data, but CCA statistics are only marginally superior for some of the other data options. Although the diatom data were transformed for DCA, they were not for CCA, even though CCA uses the same algorithm for those data. Despite lack of transformation, the CCA produced relatively smooth pH and alk reconstructions (Davis et al. 1990, 1994).

With the exception of the CCA model for untransformed alk in the 63-lake data set, the summary statistics for the three models in each of the data options are remarkably similar. This is not to say that application of the models for down-core inference will yield the same results; it does not

(Davis et al. 1990, 1994). Each model has its unique strengths and weaknesses, and for this reason, Davis et al. applied all three models at the 12 reconstruction lakes.

This study suggests that the careful selection of lakes for inclusion in calibration data sets is at least as important as the choice of regression models. The early investment of time and resources in the selection of calibration lakes that best fit paleolimnological objectives is likely to pay off with more accurate reconstructions.

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# Responses of Benthic Communities to Episodic Acid Disturbances in a Lake Outflow Stream at the Experimental Lakes Area, Ontario

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Hall, R.J. 1994. Responses of benthic communities to episodic acid disturbances in a lake outflow stream at the Experimental Lakes Area, Ontario. Can. J. Fish. Aquat. Sci. 51: 1877–1892.

Effects of episodic experimental pH depressions on water chemistry and structure of benthic communities were investigated in northwestern Ontario, a location with low $H^+$ deposition. Acidity levels were increased from background values of pH 6.9 to 5.0, 4.5, and 4.0 to simulate snowmelt and rainstorm events observed in south-central Ontario. Conductivity and concentrations of Ca and Mn increased significantly for all three pH disturbances. Fe and dissolved organic C remained unchanged at pH 4.5 and 4.0 but Fe increased at pH 5.0. Total and inorganic monomeric Al increased at pH 4.5 and 4.0 relative to reference areas. At pH 5.0, density decreases were observed for *Baetis, Ectemnia, Simulium,* and *Prosimulium,* at pH 4.5 for *Baetis, Lepidostoma, Micropsectra, Simulium,* and *Prosimulium,* and at pH 4.0 for *Baetis, Tanytarsus,* and *Cricotopus.* Densities of collector/filterers and clingers decreased at all pH levels, but diversity decreased in the experimental reach at pH 4.0 only. Drift density of the above genera was correlated with $H^+$ concentration. Large reductions in macroinvertebrate densities occurred at higher pH (5.0) and during shorter exposure times compared with previous experiments in areas of higher $H^+$ deposition in south-central Ontario and New Hampshire.

On a étudié les effets de diminutions expérimentales épisodiques du pH sur les propriétés chimiques de l'eau et la structure des populations benthiques dans le nord-ouest de l'Ontario, un endroit où les dépôts de $H^+$ sont faibles. Le niveau d'acidité a été augmenté à partir de valeurs de base de pH de 6,9 à 5,0, à 4,5 et à 4,0 de façon à simuler des événements comme la fonte des neiges et les pluies abondantes observées dans le centre-sud de l'Ontario. Les trois modifications du pH se sont accompagnées d'une augmentation significative de la conductivité et des concentrations de Ca et de Mn. Les concentrations de Fe et de C organique dissous n'ont pas varié à pH 4,5 et 4,0 mais la concentration de Fe a augmenté à pH 5,0. Les concentrations de Al monomérique total et inorganique ont augmenté à pH 4,5 et 4,0 comparativement aux régions de référence. On a observé une diminution de la densité de *Baetis,* d'*Ectemnia,* de *Simulium* et de *Prosimulium* à 5,0, de *Baetis,* de *Lepidostoma,* de *Micropsectra,* de *Simulium* et de *Prosimulium* à pH 4,5 et de *Baetis,* de *Tanytarsus* et de *Cricotopus* à pH 4,0. On a observé une diminution des organismes qui se nourrissent par filtration ou un autre mode actif et des organismes qui se fixent aux substrats à tous les pH, mais la diversité a diminué dans le bief expérimental à pH 4,0 uniquement. La densité de dérive des organismes susmentionnés était corrélée à la concentration de $H^+$. On a observé une réduction importante de la densité des macroinvertébrés à des pH plus élevés (5,0) et pour des durées d'exposition plus brèves comparativement à des expériences antérieures dans des secteurs où les dépôts de $H^+$ sont plus élevés dans le centre-sud de l'Ontario et du New Hampshire.

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Benthic communities in lake outflows in northeastern North America experience pH disturbances that result from rapidly released acidic substances during spring snowmelt and from acid soils during summer rainstorms (Hall 1990). Although episodic pH reductions and invertebrate species responses are more severe in streams draining into the poorly buffered lakes in this area (LaZerte 1984; Hall et al. 1987), significant impoverishment of biota may also occur in lake outlet streams that usually have stable chemistry. These acidic events are often of short duration, lasting only a few hours to a few days. However, long-term consequences of these episodic events may be of ecological importance, since intensity of pH disturbance is greatest in headwater lakes and streams, and these areas are sources of invertebrates for colonization downstream (Hall et al. 1982). Since lake outflows are characterized by high numbers of biota common to both lentic and lotic ecosystems, manipulations of these habitats can provide quantitative information on structure (Hall 1990) and function (Morin et al. 1988) of benthic invertebrates in streams as well as littoral areas of lakes.

Previous acid disturbance experiments were conducted in streams located in areas of high $H^+$ deposition from the atmosphere (Hall et al. 1980, 1982, 1987; Allard and Moreau 1987; Ormerod et al. 1987; Griffiths 1992), with many of the study streams having episodic pH depressions from one to three units. Only a few experiments have been conducted in lake outflows (whole-stream acidification: Hall 1990; mesocosm stream channels: Servos and Mackie 1986; Allard and Moreau 1987; Chmielewski and Hall 1992). In these lake outflow studies, pH depressions caused either no change or some change in diversity of benthic communities, with mayfly species displaying the greatest loss. Prior to these experimental studies, however, many streams in areas of

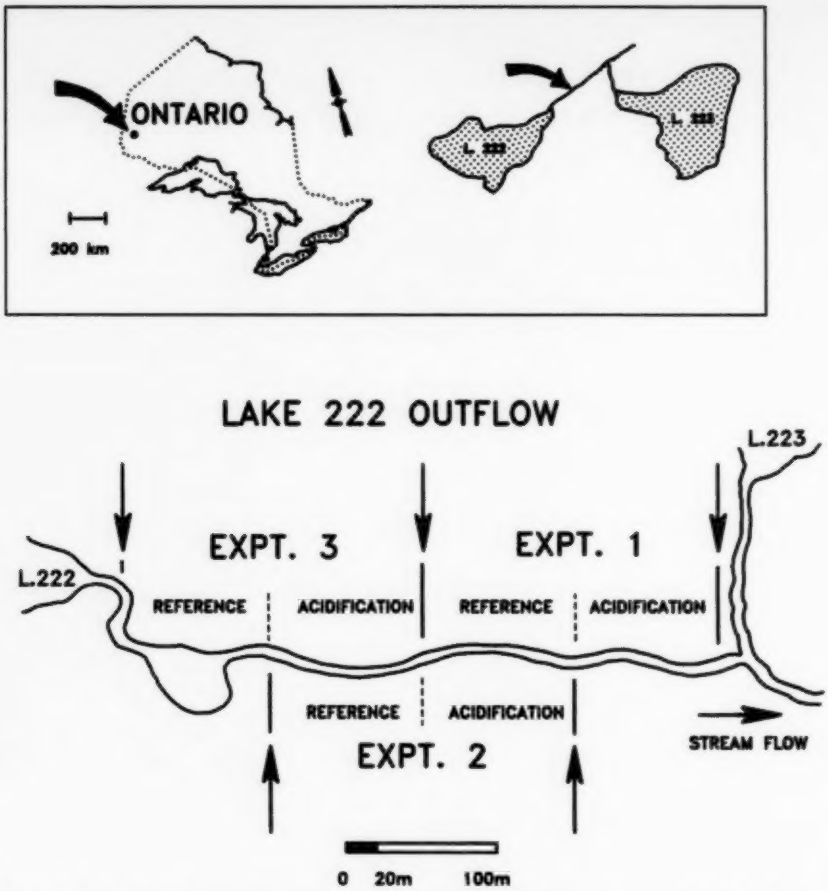

FIG. 1. Lake 222 outflow study areas for the three acidification experiments within the Experimental Lakes Area. The pH was reduced to 4.5 in experiment 1, 4.0 in experiment 2, and 5.0 in experiment 3 relative to reference values (pH 6.9). Reference areas (site A) were located 1.0 m above the acid additions. Treatment areas (sites B, C, and D) were located 15, 30, and 100 m below the acid disturbances.

high acid deposition had already displayed early warning signs of impoverished mayfly communities (Fiance 1977; Arnold et al. 1981; Kimmell et al. 1985; Mackay and Kersey 1985; Simpson et al. 1985; Smith et al. 1990; Feldman and Connor 1992; Peterson and Van Eeckhaute 1992; Rosemond et al. 1992). Thus, some physiological acclimation of organisms (McWilliams et al. 1980) or species shifts from sensitive to tolerant forms (Hall et al. 1980; Schindler et al. 1985; Hall and Ide 1987) may have already occurred.

Without quantitative data on benthic communities with previous histories of little to no exposure to $H^+$ stress, it is difficult, if not impossible, to determine whether the observed changes in benthic communities are due to acidification or to other disturbances such as drying or scouring of the stream bottom due to hydrologic fluctuations (Resh et al. 1988; Bernard et al. 1990; Hall 1990) or to elevated Al concentrations as a result of low pH (Hall et al. 1987). Thus, the goal of this investigation was to quantify ecological effects of short-term pH disturbances on lake outflow water chemistry and benthic communities in a region that is perceived to experience little to no $H^+$ deposition from anthropogenic sources. A second objective was to evaluate whether changes in pH and Al concentrations would coincide with changes in the structure of the benthic communities. Elevated Al concentrations, particularly inorganic monomeric Al (IMA), at low pH can disturb ion regulation and cause death in fish and invertebrates (Driscoll et al. 1980; Hall et al. 1985, 1987). Third, I tested the hypothesis that biotic communities not previously exposed to low pH or high Al concentrations would show greater stress when exposed to short-term pH depressions compared with benthic communities (Hall et al. 1988; Hall 1990) in areas of high acidic deposition. Here, I define greater stress as a larger change in the benthic community in response to alterations in chemistry than observed previously in similar manipulations at different locations in northeastern North America.

## Study Site

Lake 222 (93°44′W, 49°41′N) is a small headwater lake (surface area 16.4 ha, mean depth 5.8 m) located on the Canadian Shield in the district of Dryden in northwestern

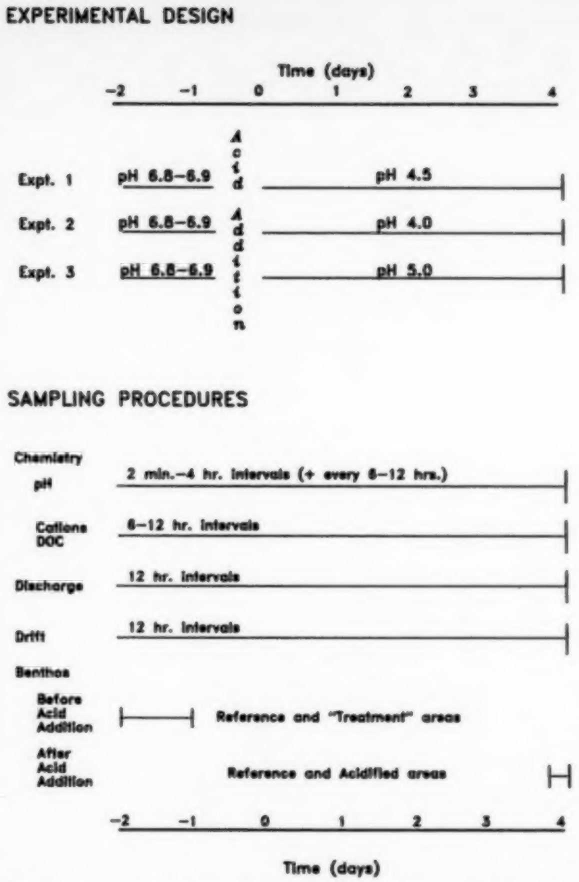

FIG. 2. Summary of experimental design and sampling procedures used for the three spring acid disturbance experiments.

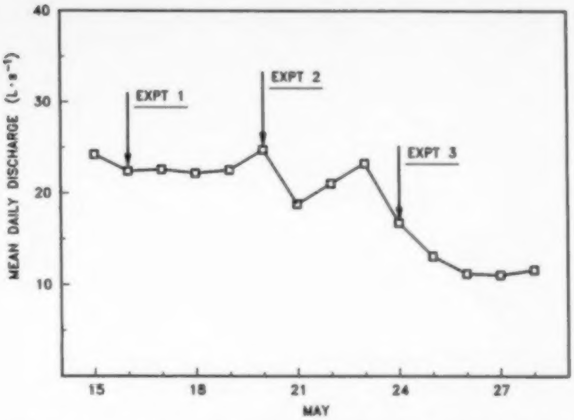

FIG. 3. Mean up- and downstream daily discharges for the spring acidification experiments. Arrows indicate the beginning of acid additions for each experiment.

Ontario (Fig. 1). Bedrock in the catchment area (204.3 ha) surrounding Lake 222 is Precambrian granite overlain in some areas by thin glacial sand and gravel (Brunskill and Schindler 1971). Jack pine (*Pinus banksiana*), black spruce (*Picea mariana*), and trembling aspen (*Populus tremuloides*) are the dominant overstory riparian tree species. The outflow of Lake 222 joins with Lake 223 outflow 450 m downstream (Fig. 1). A 400-m reach in which elevation dropped 8.1 m was used for experiments. The stream channel in the study area was approximately 1 m wide. The bottom consisted of coarse sand and gravel with large boulders scattered throughout. During the study, water temperature was 10.0–15.3°C ($\bar{X}$ ± SE ($N$) = 12.5 ± 2.7°C (22)) in experiment 1, 9.0–15.8°C (13.4 ± 2.9°C (22)) in experiment 2, and 11.8–18.0°C (15.1 ± 3.2°C (22)) in experiment 3.

## Methods

### pH Disturbance Experiments

Three experiments were conducted between May 14 and 28, 1983. Each experiment utilized 200 m of the 400-m study area, starting with the lowermost 200 m in the first experiment and moving 100 m upstream for each of the succeeding two experiments. The upstream 100-m reference area of one experiment became the downstream 100-m treated area of the succeeding experiment (Fig. 1). Collections of samples for streamwater chemistry, invertebrate drift, and benthic communities were made above and below the acid injection location at four stations for each experiment. Sampling stations were located 1.5 m (site A, reference) above and 15 (site B), 30 (site C), and 100 m (site D) below acidification for each experiment.

To achieve pH depressions similar to those used in experiments conducted in New Hampshire and Dorset, Ontario (Hall et al. 1987; Hall 1990), dilute concentrations (0.05–1.0 N) of sulphuric and hydrochloric acids (reagent grade) were mixed in a 300-L carboy and added to the stream. The drip rate of acid into the stream was adjusted by a peristaltic pump and a Teflon stem needle valve in borosilicate. The pH was reduced at 4.5 in experiment 1, to 4.0 in experiment 2, and to 5.0 in experiment 3 (Fig. 2). In each experiment, pH reduction lasted 4 d. pH was monitored 4 m below acid addition every 2 min to 4 h depending on discharge.

Discharge was calculated by integrating information on velocity, depth, and width for each experiment. Transects were laid from bank to bank above site A and below site C. Current velocity was measured at 12-h intervals with a Teledyne Gurley current meter at 0.6 times the water depth at points 10–20 cm along each transect. Mean upstream/downstream discharge ($\bar{X}$ ± SE ($N$)) was 23.1 ± 0.43 $L \cdot s^{-1}$ (48) in experiment 1, 19.2 ± 1.6 $L \cdot s^{-1}$ (44) in experiment 2, and 13.6 ± 1.8 $L \cdot s^{-1}$ (44) in experiment 3 (Fig. 3).

### Water Chemistry

For each experiment, water samples were collected at all four stations every 12 h for 2 d prior to and during 4 d of acid addition (Fig. 2). At each station, water samples were collected in two acid-washed 500-mL polystyrene bottles and one 250-mL brown nalgene bottle. Ca, Fe, Mn, and dissolved organic C (DOC) were measured in one 500-mL sample; total Al, IMA, and Mn were measured in the other 500-mL sample. DOC was measured colorimetrically after the sample was oxidized in a UV digestor in an acid–persulphate medium. Ca, Fe, and Mn were analyzed by flame atomic absorption. pH was analyzed from water in the 250-mL nalgene bottle with a combination glass electrode standardized against appropriate buffers. pH was measured within a few hours whereas other chemical parameters were determined within a week. Detailed methods for chemical analyses are described elsewhere (MOE 1983; Locke and Scott 1986). Al speciation methods followed those outlined by LaZerte (1984).

### Drift and Benthic Studies

Drift nets were placed side by side extending from one stream bank to the other at four sampling stations (sites A, B, C, and D) to determine what immature aquatic insects were most sensitive to increased acidity. Each net was constructed of a polyvinylchloride frame (15 × 30 cm) to which was attached a collecting bag (1 m long) made of Nitex (mesh opening 273 μm) and was anchored in the stream by wooden dowels driven into the substrate. Drift nets also were placed from bank to bank 100 m upstream of acid addition to block drifting invertebrates; thus, equal areas above and below pH disturbance were studied (Fig. 1). For each experiment, drift nets were removed and immediately replaced with clean ones every 12 h for 2 d before and 4 d during experimental pH depressions (Fig. 2). At each station, contents of all drift nets extending from bank to bank from the two 12-h periods were combined. Drift samples were preserved in 10% formalin. Numbers of individuals collected were converted to numbers per 100 $m^3$ per day.

A cylindrical sampler enclosing 0.0314 $m^2$ of stream sediment was used to determine quantitative differences between benthic communities in reference and acidified reaches for the three experiments. For each experiment, 15–20 bottom samples were collected from riffle areas in each 100-m reference and treatment area before acid disturbance and 15–20 samples in each of the same two study areas after the 4-d pH reduction via a stratified random design (Fig. 2). The sampler was placed into the streambed to a depth of 15 cm. Sediments were repeatedly hand agitated and suspended organisms were collected with a hand net until no more organisms were observed. One centimetre of surface sediments in each core was collected to capture caddisflies and molluscs that had settled quickly following agitation. Samples were preserved in 10% formalin. Numbers of insects were multiplied by 31.8 to convert to number of invertebrates per square metre.

In the laboratory, drift and bottom samples were washed through an elutriator (Stewart 1975), as well as through a series of graduated sieves (0.6–0.01 mm aperture size). Organisms were subsampled where appropriate (Hall 1990) and identified and enumerated. Taxomonic organization into functional and behavioural guilds followed classifications of Merritt and Cummins (1986).

### Diversity

From results of previous studies, I hypothesized that diversity would be reduced during the three pH treatments because the benthic communities have not been preexposed to drastic pH depressions. Diversity was estimated using abundances at the generic level of taxomonic resolution and is another measure of how the community responded to the pH depressions. Generic diversities were computed for each benthic sample using Brillouin's formula ($H$; Lloyd et al. 1968; Pielou 1975). Evenness ($J$) was also calculated to determine equitability of abundance of genera in each sample.

### Statistical Analyses

Invertebrate communities collected daily in drift samples were identified using correspondence analysis (CA). This procedure (also called reciprocal averaging; Gauch et al. 1977) determines sample scores and taxa scores without prior grouping of the samples. Therefore, the drift communities are evaluated independently of the information about sample location (Ludwig and Reynolds 1988). The CA technique is similar to principal components and factor analysis, but is superior to other ordinations for describing environmental patterns (Kenkel and Orloci 1986; Matthews et al. 1991). In a previous study (Hall 1990), I used nonmetric multidimensional scaling (NMDS) to describe patterns in the drift community; however, NMDS tends to merge groups and does not provide estimates of the percentage of the total variance in each axis, or simple ways to assess the contributions of the various taxa to the NMDS axes. By contrast, CA maintains discontinuities if they exist and provides more information to assist in the interpretation of the ordination plots. I compared the drift community results from this study using both CA and NMDS and concluded that CA was superior in producing biological summaries that resembled the environmental (e.g., discharge, chemistry) patterns. CA is also preferred over detrended CA (Jackson and Somers 1990).

There were 72 drift samples in total (4 sites × 6 d × 3 experiments). Only the most common species (representing ≥5% of total drift community) were used in the analyses because rare taxa can distort ordination results (ter Braak and Prentice 1988). The drift communities comprising 25 or 26 species were summarized into three CA ordination axes (Rohlf 1988). Spearman's rank correlations were then computed between the first three CA axis scores and a number of environmental variables to reveal environmental correlates with the dominant trends in the drift community. Similarly, rank correlations between CA scores and the taxa comprising the drift samples were also calculated to identify those genera that contributed the largest variation to the CA drift community. The ordination and the subsequent correlation analysis were completed using both the raw numbers and discharge-corrected data (i.e., drift density as number per 100 $m^3$ per day) to evaluate the influence of adjusting for different stream flow rates on the interpretation of the results. Since no difference in these results was apparent, only the ordination scores for discharge-corrected drift are reported here.

A Student $t$-test was used to test for differences in mean chemical concentration and mean drift density between paired samples partitioned into taxonomic, functional, and behavioural groups (i.e., site A versus B, site A versus C, site A versus D) for the three experiments.

Statistical analyses of the benthic communities were patterned after the optimal impact study design proposed by Green (1979). Replicate samples were collected at sites A and B before short-term pH disturbance and at the same two locations 4 d after pH disturbance, with pH reduction occurring at site B only. Benthic data were transformed using log $(x + 1)$ to remove observed heterogeneity of error variances. I used a two-way (areas-by-times) factorial ANOVA or MANOVA design (Wilkinson 1988) to test the null hypothesis that no change in benthic communities (total numbers, numbers within behavioural, functional, and taxonomic groups, or numbers within genera) occurred in the pH-disturbed areas using the null hypothesis of no

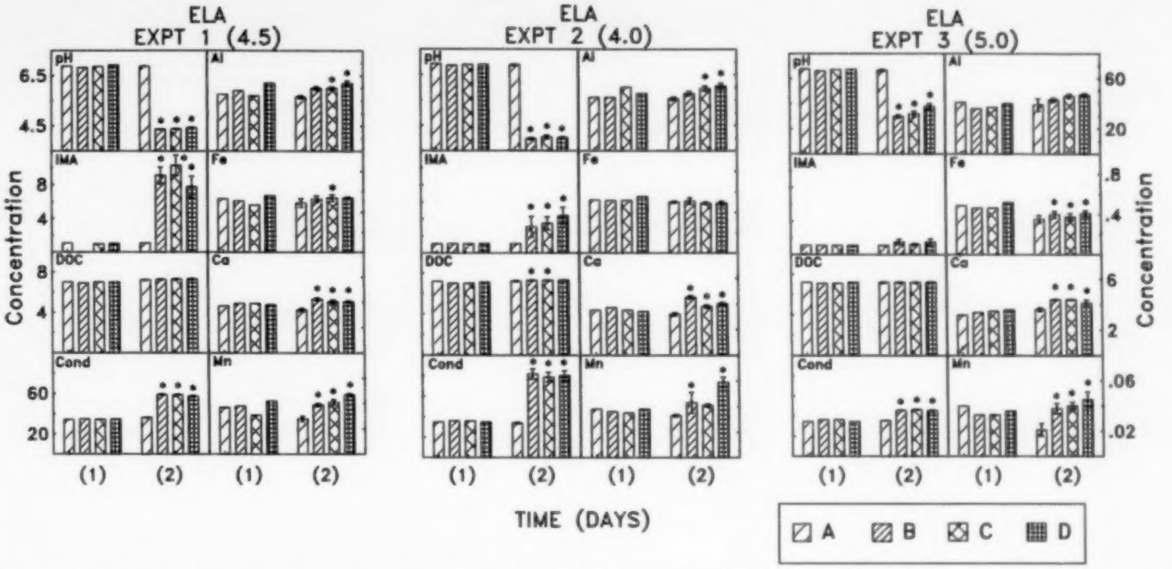

FIG. 4. Daily water chemistry of the stream prior to and during the acid disturbance experiments. Histograms depicting abiotic parameters prior to the treatment (time = (1)) represent means for two samples at sites A, B, C, and D, and during the experiment, means (±SE, $N = 8$) for the 4-d treatments (time = (2)). The pH during experiment 1 was reduced to 4.5, 4.0 in experiment 2, and 5.0 in experiment 3. Concentrations for Fe, DOC, Ca, and Mn are $\mathrm{mg \cdot L^{-1}}$ and for total Al and IMA are $\mathrm{\mu g \cdot L^{-1}}$, and conductivity = $\mathrm{\mu S \cdot cm^{-1}}$.

areas-by-times interaction. A significant interaction between space and time revealed that a difference between sites was present after acid addition using either a univariate or a multivariate ANOVA (see Results). If a significant interaction was demonstrated, I then used a one-way ANOVA or MANOVA for each time (before and after) to determine if differences in benthic communities occurred in the two study areas (reference and treatment) before pH disturbance and how these differences between the same two areas changed after the pH manipulation. Pseudo-replication was a potential problem with this study design (Hurlbert 1984); thus, differences between reference and treatment areas may not be attributed solely to pH treatment effects.

## Results

### Stream Chemistry

During acid disturbance, mean pH ranged from 6.83 to 6.88 in the three reference areas (site A, Fig. 4). Sites B, C, and D for experiments 1 and 2 had similar mean pH values (4.5 and 4.0, respectively) during each acidification experiment. However, in experiment 3 (pH 5.0), neutralization of added acid took place at site D (Fig. 4), producing a pH higher at site D relative to site B.

Conductivity and concentrations of Ca and Mn increased significantly ($P < 0.05$, paired $t$-test) in treatment areas relative to reference sites during the three pH disturbance experiments (Fig. 4) whereas total Al increased ($P < 0.05$) only at pH 4.5 and 4.0. Concentrations of Fe were significantly higher at sites B–D relative to site A for experiment 3 (pH 5.0) and at one site (C) in experiment 1. DOC increased significantly in experiment 2 (pH 4.0) at sites B and C only. No increase was detected for IMA and total Al in experiment 3 (mean pH 5.0), but significant ($P < 0.05$) increases in total Al (sites C and D) and IMA concentrations were observed in experiments 1 (pH 4.5) and 2 (pH 4.0).

### Benthic Community

For all three experiments, a significant interaction ($P < 0.05$, two-way ANOVA) occurred between mean total benthic density at sites A and B before and after acidification (Table 1), indicating that differences between reference (A) and treatment (B) sites after acid disturbance changed from those before treatment. One-way ANOVA showed no differences in benthic density ($P > 0.05$) between sites A and B for all three experiments before acid addition; however, significant ($P < 0.05$) reductions in density were apparent after acid addition at site B relative to site A (Table 1).

Partitioning benthic communities into taxonomic categories revealed significant interactions ($P < 0.05$, two-way ANOVA) and significant decreases in mean density ($P < 0.05$, one-way ANOVA) after acid disturbance for dipterans in the three experiments (Fig. 5, 6, and 7), for ephemeropterans in experiment 2 (Fig. 6), and for miscellaneous invertebrates in experiment 3 (Fig. 7). No significant multivariate interaction between space and time occurred for the dominant taxa in the benthic community (Table 2) in experiment 1 (Wilks' lambda = 0.74; approximate $F = 0.778$; df = 21,47; $P = 0.73$) or experiment 2 (Wilks' lambda = 0.76; approximate $F = 0.766$; df = 21,52; $P = 0.74$), but did occur in experiment 3 (Wilks' lambda = 0.308; approximate $F = 4.797$; df = 22,47; $P < 0.001$).

No difference in density for the dominant benthic taxa was evident between reference and treatment areas before acid disturbance in any of the three experiments ($P > 0.05$, one-way ANOVA; Table 1). Following pH reduction to 4.5 in experiment 1, significant ($P < 0.05$, ANOVA) reductions were evident in the treatment area for *Simulium*, *Micropsectra*,

TABLE 1. Mean number of individuals·$m^{-2}$ (±SE), genetic diversity (*H*), and evenness (*J*) of invertebrates collected in spring (14–28 May) with a 0.0314-$m^2$ benthic cylinder sampler. Mean pH values for treatment sites were 4.5 (experiment 1), 4.0 (experiment 2), and 5.0 (experiment 3). Mean pH levels in the reference area were similar (6.9) for all three experiments. Asterisks represent $P < 0.05$ (one-way ANOVA). Two-way ANOVA analysis showed significant interaction ($P < 0.05$).

| | Before acid | | After acid | |
|---|---|---|---|---|
| | Reference (A) ($m^2$) | Treatment (B) ($m^2$) | Reference (A) ($m^2$) | Treatment (B) ($m^2$) |
| Experiment 1 (pH 4.5) | | | | |
| Total number of individuals | 14 105 (5004) | 14 755 (5543) | 17 600 (4830) | 5824.5 (594.7)* |
| Number of genera | 9.7 (1.25) | 10.0 (0.70) | 9.9 (1.3) | 5.8 (0.7)* |
| *H* | 2.3 (0.16) | 2.09 (0.19) | 2.05 (0.17) | 1.8 (0.16) |
| *J* | 0.82 (0.03) | 0.70 (0.03)* | 0.79 (0.03) | 0.8 (0.03) |
| *N* | 18 | 17 | 18 | 18 |
| Experiment 2 (pH 4.0) | | | | |
| Total number of individuals | 13 500 (5018.5) | 16 513 (4858) | 13 213 (5215) | 6309 (1650)* |
| Number of genera | 9.6 (1.2) | 8.3 (0.86) | 10.8 (1.5) | 6.3 (1.3)* |
| *H* | 1.97 (0.21) | 1.67 (0.15) | 2.2 (0.19) | 1.48 (0.21)* |
| *J* | 0.67 (0.034) | 0.72 (0.04) | 0.76 (0.04) | 0.77 (0.05) |
| *N* | 19 | 18 | 18 | 20 |
| Experiment 3 (pH 5.0) | | | | |
| Total number of individuals | 12 927 (21 445) | 14 439 (2042) | 16 146 (5272) | 5545 (594.7)* |
| Number of genera | 9.4 (1.4) | 5.2 (0.57)* | 5.5 (0.6) | 5.3 (1.2) |
| *H* | 2.0 (0.18) | 1.45 (0.13)* | 1.5 (0.15) | 0.15 (0.24) |
| *J* | 0.71 (0.03) | 0.67 (0.05) | 0.67 (0.05) | 0.72 (0.06) |
| *N* | 19 | 18 | 16 | 19 |

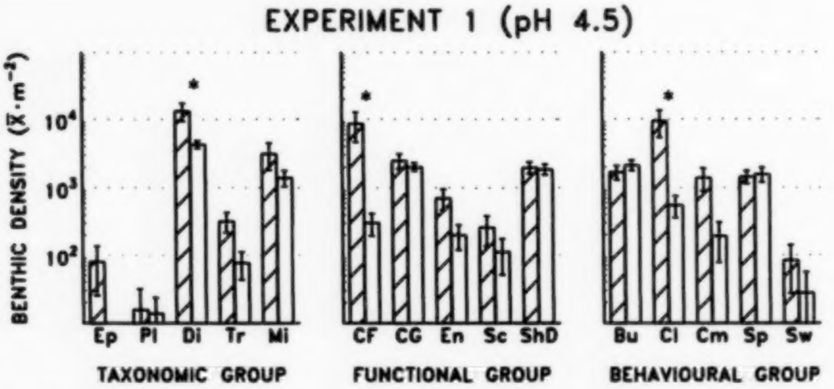

FIG. 5. Benthic density ($\bar{X}$ ± SE, $N = 17–18$) in reference (hatched bars) and treatment areas (open bars) after pH disturbance (experiment 1, pH 4.5) in spring (May) by order (Ep = Ephemeroptera, Pl = Plecoptera, Di = Diptera, Tr = Trichoptera, Mi = miscellaneous), functional group (CF = collector/filterers, CG = collector/gatherers, En = engulfers, Sc = scrapers, ShD = shredders), and behavioural group (Bu = burrowers, Cl = clingers, Cm = climbers, Sp = sprawlers, Sw = swimmers). Asterisks = $P < 0.05$, one-way ANOVA.

*Prosimulium*, *Lepidostoma*, and *Baetis*, and an increase was noted for *Tanytarsus* (Table 2). After pH reduction to 4.0 in experiment 2, significant ($P < 0.05$, ANOVA) decreases in densities were found for *Tanytarsus*, *Cricotopus*, and *Baetis* whereas in experiment 3 (pH reduction to 5.0), decreases after acid disturbance were observed in *Simulium*, *Prosimulium*, and *Ectemnia* (Table 2). In summary, all of the above taxa (seven genera) that showed reductions in density are considered sensitive to short-term pH disturbances whereas the remaining 23 genera appear tolerant (Table 2).

### Generic Richness, Diversity, and Evenness

A significant ($P < 0.05$, two-way ANOVA) interaction was found for generic richness in experiments 1 (pH 4.5) and 2 (pH 4.0) and evenness in experiment 1. A one-way ANOVA indicated significant differences ($P < 0.05$) after

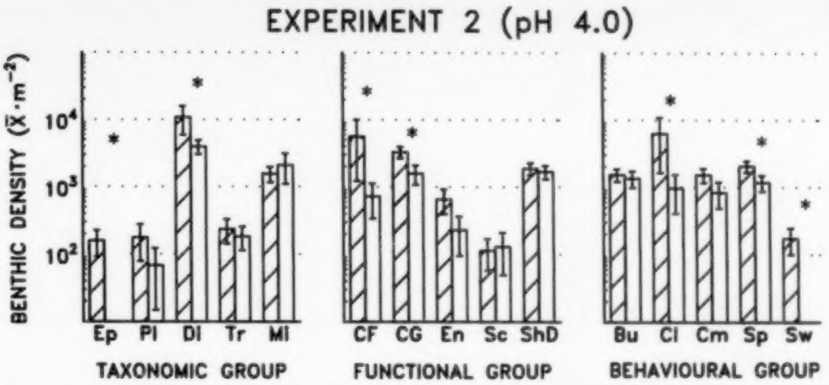

FIG. 6. Benthic density ($\bar{X}$ ± SE, $N = 18–20$) in reference (hatched bars) and treatment areas (open bars) after pH disturbance (experiment 2, pH 4.0) in spring (May). See Fig. 5 legend for details.

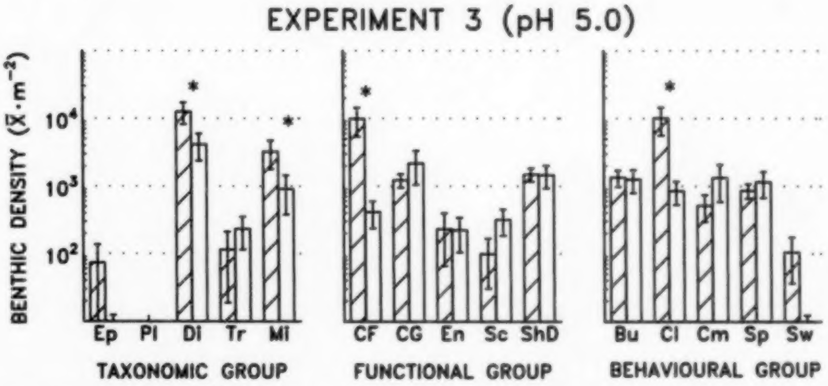

FIG. 7. Benthic density ($\bar{X}$ ± SE, $N = 16–19$) in reference (hatched bars) and treatment areas (open bars) after pH disturbance (experiment 3, pH 5.0) in spring (May). See Fig. 5 legend for details.

acidification for generic richness in experiments 1 and 2 and diversity in experiment 2, suggesting a decline associated with low pH. In experiments 1 and 3 (pH 5.0), significant interaction ($P < 0.05$) for evenness, generic richness, and diversity was due to significant differences before acidification (Table 1).

### Individual and Community Level Patterns in the Invertebrate Drift

Acid disturbance resulted in variable responses in the invertebrate drift relative to the untreated reference areas (Fig. 8, 9, and 10). Significant ($P < 0.05$) increases in the drift of dipterans occurred at sites C and D in experiment 1 (Fig. 8) and at sites B, C, and D in experiments 2 (Fig. 9) and 3 (Fig. 10). Ephemeropterans increased ($P < 0.05$) in the drift at site D compared with site A for all three acid disturbance experiments whereas trichopterans increased ($P < 0.05$) in the drift at sites B and C in experiment 3 (Fig. 10). In contrast, invertebrates within the miscellaneous category significantly ($P < 0.05$) decreased at site B in experiment 1 (Fig. 8) and at sites C and D in experiment 2 (Fig. 9).

The eigenvalues for the first three axes of the correspondence analyses accounted for up to 69% (experiment 1, 59.3%; experiment 2, 64.7%; experiment 3, 69%) of the variation in the drift among the 72 samples (Tables 3, 4, and 5). Individual taxa in the drift that were associated with each axis (Tables 3, 4, and 5) are ranked from those species representing the largest portion of the total (e.g., *Simulium*) to those representing the least proportion (e.g., *Leptophlebia*, *Eukiefferiella*, and *Dixa*, respectively, for the three experiments). Sensitive taxa that increased proportionately ($P < 0.05$) in the drift are those displaying large rank correlations with signs opposite to that displayed by pH for that axis (Table 6). These taxa were *Baetis*, Acarina, *Brachycentrus*, *Procladius*, Cladocera, *Eukiefferiella*, and Oligochaeta in experiment 1 (axes 1 and 2 only). The list extends in experiment 2 (pH reduction to 4.0) to include *Simulium*, *Ectemnia*, *Cricotopus*, *Thienemanniella*, *Tanytarsus*, as well as *Baetis* and *Eukiefferiella* larvae. Those species affected by pH reduction to 5.0 in experiment 3 nearest to the lake outflow were *Simulium*, *Ectemnia*, *Sphaerium*, *Hyallela*, Acarina, *Baetis*, *Leptophlebia*, *Procladius*, *Tanytarsus*, *Macronema*, *Hydroscapha*, and Chironomini and Pentaneurini pupae. In contrast, the proportion of *Prosimulium* in the drift significantly ($P < 0.05$) decreased with reduced pH (rank correlation numbers with signs similar to that for pH) for axis 2 in experiment 2 and axis 1 in experiment 3.

TABLE 2. Mean number of individuals·m$^{-2}$ (±SE) of the dominant taxa collected from Lake 222 in spring with a 0.0314-m$^2$ benthic cylinder sampler. Mean treatment pH values were 4.5 (experiment 1), 4.0 (experiment 2) and 5.0 (experiment 3); reference pH levels were 6.80–6.95. Asterisks represent significance levels ($P < 0.005$, one-way ANOVA, log $(x + 1)$ transformed). N = nymphs, L = larvae, P = pupae; CG = collector/gatherers, ShD = shredders, CF = collector/filterers, En = engulfers, Sc = scrapers; Sw = swimmers, Sp = sprawlers, Bu = burrowers, Cm = climbers; Cl = clingers.

| | Lifestage/ functional/ behavioural group | Experiment 1 (4.5) | | Experiment 2 (4.0) | | Experiment 3 (5.0) | |
|---|---|---|---|---|---|---|---|
| | | Reference | Treatment | Reference | Treatment | Reference | Treatment |
| Sensitive | | | | | | | |
| Ephemeroptera | | | | | | | |
| *Baetis* | N/CG/Sw | 61.9 (53) | 0* | 146.6 (65.7) | 0* | 63.6 (61.5) | 0 |
| Trichoptera | | | | | | | |
| *Lepidostoma* | L/ShD/Sp | 256.2 (98.3) | 28.3 (18.8)* | 70.6 (32.2) | 79.5 (38.4) | 95.4 (92.3) | 95.4 (57.7) |
| Diptera | | | | | | | |
| *Tanytarsus* | L/CG/Bu | 498.2 (155.5) | 1166 (192.3)* | 899.2 (233.7) | 343.4 (172.9)* | 524.7 (194) | 557.3 (312.3) |
| *Micropsectra* | L/CG/Cm | 1413 (502.6) | 176.7 (112.7)* | 1510.5 (354.5) | 831.5 (340.4) | 508.8 (216) | 1332 (735.9) |
| *Cricotopus* | L/ShD/Cl | 413.4 (176.3) | 127.2 (63.2) | 341 (211.4) | 46.1 (40.3)* | 127.2 (84.3) | 117.2 (104.2) |
| *Simulium* | L/CF/Cl | 7222.2 (3376) | 261.5 (97)* | 3621.7 (2938) | 470.6 (267.3) | 8745 (3679) | 398 (165.3)* |
| *Prosimulium* | L/CF/Cl | 994.6 (351.2) | 28.3 (27.5)* | 1475 (1228) | 36.6 (24.9) | 668 (372.8) | 1.7 (1.6)* |
| *Ectemnia* | L/CF/Cl | 424 (330.2) | 0 | 0 | 81.1 (79) | 493 (369) | 0* |
| Tolerant | | | | | | | |
| Ephemeroptera | | | | | | | |
| *Leptophlebia* | N/CG/Sw | — | — | — | — | 9.9 (7.8) | 5.0 (2.7) |
| Plecoptera | | | | | | | |
| *Leuctra* | N/ShD/Cl | — | — | 56.5 (54.9) | 0 | — | — |
| *Allocapnia* | N/ShD/Cl | — | — | 84.8 (59.9) | 0 | — | — |
| Trichoptera | | | | | | | |
| *Polycentropus* | L/En/Cl | — | — | — | — | 9.9 (2.3) | 0 |
| *Platycentropus* | L/ShD/Cm | — | — | — | — | 9.9 (4.6) | 6.7 (4.2) |
| *Macronema* | L/CF/Cl | — | — | — | — | 4.0 (2.6) | 16.7 (14.5) |
| *Neophylax* | L/Sc/Cl | — | — | — | — | 4.0 (2.6) | 3.4 (2.5) |
| *Hydropsyche* | L/CF/Cl | — | — | — | — | 2.0 (1.9) | 0 |
| Diptera | | | | | | | |
| *Ablabesmyia* | L/En/Sp | 178.4 (121.2) | 7.0 (6.9) | 591.8 (277.4) | 456.3 (163.6) | 31.8 (30.8) | 107.1 (104.2) |
| *Procladius* | L/En/Sp | 199.6 (106.6) | 227.9 (123) | 40.6 (32.9) | (76.3) (54.2) | 381.6 (159) | 354.8 (145.6) |
| *Psectrocladius* | L/CG/Sp | — | — | — | — | 31.8 (30.8) | — |
| *Natarsia* | L/En/Sp | 335.7 (139) | 684.4 (241) | 720.8 (208) | 232 (109.3) | 286.2 (110) | 237.7 (161.2) |
| *Paracladopelma* | L/?/Sp | 192.6 (123) | 153.7 (114.6) | 70.6 (39.8) | 154.2 (101) | — | — |
| *Pseudochironomus* | L/CG/Bu | 74.2 (40) | 7.0 (6.9) | 157.2 (86.7) | 132 (70.4) | 31.8 (31) | 0 |
| *Microtendipes* | L/CF/Cl | 97.2 (65.8) | 0 | — | — | — | — |
| *Rheotanytarsus* | L/CF/Cl | — | — | 462.8 (258) | 76.3 (37.7) | — | — |
| *Thienemanniella* | L/CG/Sp | 95.4 (57.5) | 28.3 (18.8) | 185.5 (74.7) | 27 (17) | 0 | 6.7 (6.5) |
| *Corynoneura* | L/CG/Sp | 86.6 (61.5) | 14.1 (13.7) | — | — | — | — |
| *Eukiefferiella* | P/CG/Sp | 0 | 113 (109.9) | — | — | 0 | 53.5 (52) |
| *Parametriocnemus* | L/CG/Sp | 30 (27.4) | 339.2 (159.9) | 150.2 (78.7) | 14.3 (14) | 31.8 (30.8) | 80.3 (56.9) |
| *Culicoides* | L/En/Bu | 1127 (358.7) | 968 (276.7) | 431 (101.6) | 869.7 (244.4) | 795 (340) | 708 (206.2) |
| Miscellaneous | | | | | | | |
| *Stenelmis* | ?/Sc/Cl | 259.7 (123.5) | 113 (60.7) | 81.3 (47.6) | 119.3 (70.2) | 95.4 (67) | 204.2 (90) |

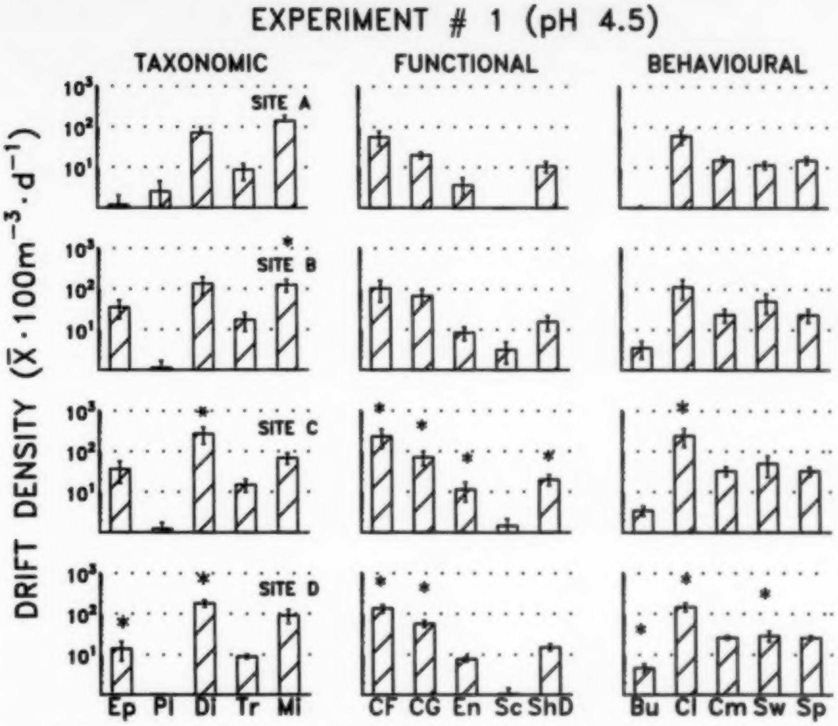

FIG. 8. Drift density ($\bar{X}$ ± SE, $N = 4$) for experiment 1 (pH 4.5) at sites A (1.5 m above), B (15 m below), C (30 m below), and D (100 m below acid addition) in spring by order (Ep = Ephemeroptera, Pl = Plecoptera, Di = Diptera, Tr = Trichoptera, Mi = miscellaneous), functional group (CF = collector/filterers, CG = collector/gatherers, En = engulfers, Sc = scrapers, ShD = shredders), and behavioural group (Bu = burrowers, Cl = clingers, Cm = climbers, Sp = sprawlers, Sw = swimmers). Asterisks = $P < 0.05$, paired $t$-test.

Spearman's rank correlations between the site scores determined by CA for the drift samples and the measured chemical and physical variables (Table 6) identified the most important environmental factors that produced a response for the entire drift community. pH (and the associated significant chemical changes with a sign opposite to that for pH) accounted for most of the variation in the first and second axes of the drift community in experiments 1 and 2 and for the first axes in experiment 3 (Table 6). Variation in the third axis correlated with conductivity (experiment 1) and total Al (experiment 2). No significant correlations between drift and the environmental variables were evident for axes 2 and 3 in experiment 3 (pH 5.0).

### Drift and Benthic Invertebrate Comparison

In general, the invertebrate drift exhibited greater changes in response to acid disturbance than the benthic invertebrate density. Direct comparison of drift responses as a result of acid disturbance is complicated by the distance below the lake outflow. More invertebrates were caught in the drift in experiment 3 (Table 5), close to the lake outflow, than in experiment 1 (Table 3), farthest from the outflow. Taxa within the drift community of experiment 3 consisted of a combination of those whose habitat is primarily the shallow littoral area of the lake (e.g., *Hyallela*, *Procladius*, *Hydroscapha*, and Chironomini and Pentaneurini pupae) as well as the stream filter-feeders (e.g., *Simulium*, *Ectemnia*, *Sphaerium*, and *Macronema*). More taxa were likely collected in the drift than benthic samples because the entire water column from bank to bank was filtered continuously with the former method. Thus, a greater variety of taxa from different microhabitats (e.g., riffles pools, leaf litter accumulations, sandy sediments, etc.) would be captured in drift nets than bottom samples that were primarily collected from riffle habitats. Despite different sampling procedures, some taxa displayed predictable responses. For example, the mayfly *Baetis* increased ($P < 0.05$) in the drift of three experiments and decreased ($P < 0.05$) in density in two experiments. The blackfly *Simulium* and the midge *Tanytarsus* increased in the drift ($P < 0.05$) and decreased in density in two of three acid disturbance experiments. The chironomid genera *Eukiefferiella* and *Procladius* increased ($P < 0.05$) in two of the drift experiments but did not show significant decreases in density. Lastly, the blackfly *Ectemnia* increased ($P < 0.05$) in drift in two experiments but decreased ($P < 0.05$) in density relative to the reference area in only one experiment (3).

### Functional and Behavioural Groups

Decreases in benthic densities in acid-disturbed sites relative to reference areas were primarily due to the collector/filterer functional group for all three experiments (Fig. 5, 6, and 7) and collector/gatherers for only experiment 2

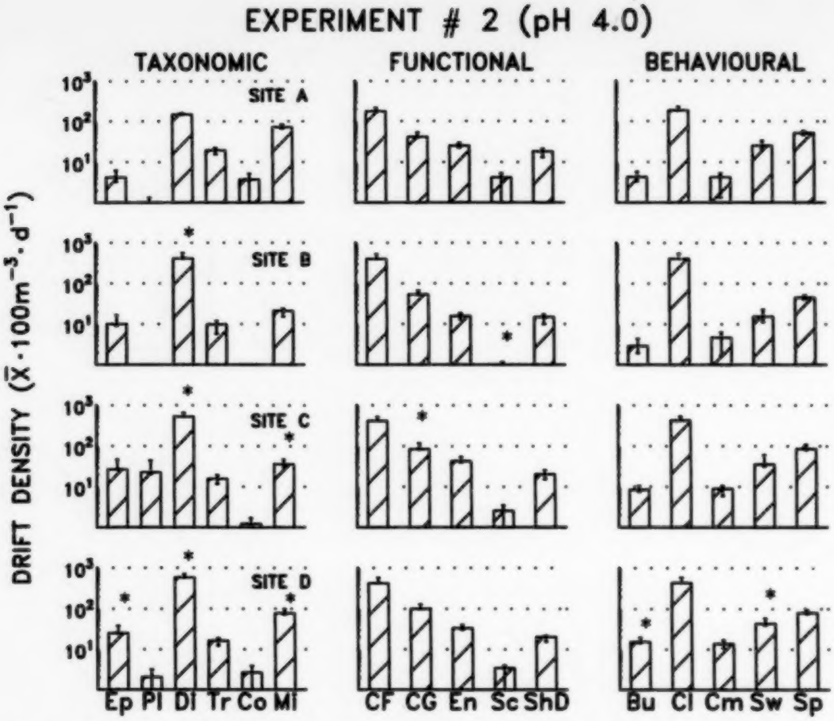

FIG. 9. Drift density ($\bar{X}$ ± SE, $N = 4$) for experiment 2 (pH 4.0). See Fig. 8 legend for details.

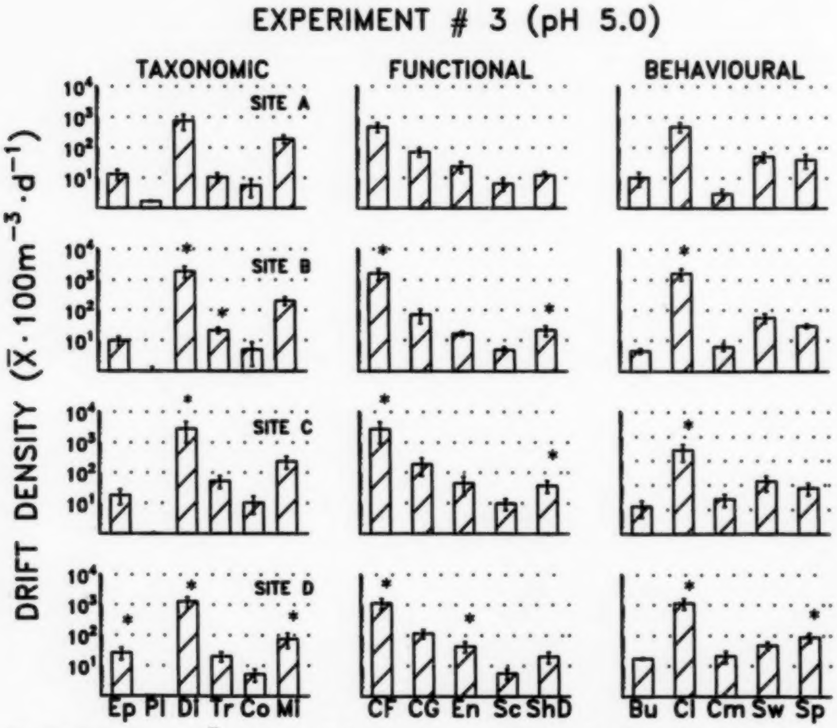

FIG. 10. Drift density ($\bar{X}$ ± SE, $N = 4$) for experiment 3 (pH 5.0). See Fig. 8 legend for details.

**TABLE 3.** Spearman's rank correlation between invertebrate drift communities (number·100 $m^{-3}·d^{-1}$) at sites A, B, C, and D and CA axes for acidification experiment 1. Asterisks indicate that absolute value $|r| > 0.40$ is significantly different from zero at the 5% level with the Bonferroni correction (Cooper 1968).

| | Life stage/ functional/ behavioural group[a] | Experiment 1 (pH 4.5) | | |
|---|---|---|---|---|
| | | CA1 | CA2 | CA3 |
| *Simulium* | L/CF/Cl | −0.210 | −0.011 | −0.251 |
| *Prosimulium* | L/CF/Cl | −0.008 | −0.314 | −0.791* |
| *Baetis* | N/CG/Sw | −0.554* | −0.666* | 0.016 |
| *Ectemnia* | L/CF/Cl | −0.196 | −0.051 | −0.293 |
| *Lepidostoma* | L/ShD/Sp | −0.321 | −0.066 | 0.027 |
| *Cricotopus* | L/ShD/Cl | −0.092 | −0.050 | −0.309 |
| *Micropsectra* | L/CG/Cm | −0.238 | 0.247 | 0.425* |
| Acarina | ?/CG/Sw | −0.425* | 0.094 | −0.524* |
| *Dixa* | L/CG/Sw | −0.038 | −0.154 | −0.371 |
| *Prosimulium* | P/CF/Cl | 0.370 | −0.138 | −0.402* |
| *Ablabesmyia* | L/En/Sp | −0.199 | 0.065 | 0.043 |
| *Natarsia* | L/En/Sp | 0.010 | 0.039 | 0.224 |
| *Brachycentrus* | L/CF/Cl | −0.559* | 0.011 | −0.447* |
| *Sphaerium* | ?/CF/Cl | 0.426* | 0.144 | 0.051 |
| *Thienemanniella* | L/CG/Sp | −0.361 | 0.277 | 0.308 |
| *Procladius* | L/En/Sp | 0.253 | 0.469* | 0.288 |
| Cladocera | ?/CG/Sw | −0.605* | 0.453* | −0.178 |
| *Hydatophylax* | L/ShD/Sp | −0.285 | 0.173 | 0.00 |
| Orthocladiini | P/CG/Sp | 0.241 | 0.140 | −0.076 |
| *Nectopsyche* | L/ShD/Cm | −0.015 | −0.308 | −0.661* |
| *Eukiefferiella* | P/CG/Sp | −0.348 | 0.527* | 0.431* |
| Oligochaeta | ?/CG/Bu | −0.412* | 0.327 | −0.432* |
| *Tanytarsus* | L/CG/Bu | 0.198 | 0.291 | 0.298 |
| Nematoda | ?/CG/Cl | 0.138 | −0.125 | −0.157 |
| *Glossosoma* | L/Sc/Cl | −0.183 | 0.106 | −0.388 |
| *Leptophlebia* | N/CG/Sw | 0.221 | −0.005 | 0.076 |
| Eigenvalue | | 0.2095 | 0.1063 | 0.0761 |
| Percent | | 31.74 | 16.09 | 11.52 |
| Cumulative | | 31.74 | 47.83 | 59.35 |

[a] See Table 2 for definitions.

(Fig. 6; $P < 0.05$, one-way ANOVA). Significant reductions in densities ($P < 0.05$) in the acid-disturbed areas of those individuals that are considered clingers were evident for all three experiments, while significant decreases in density also occurred for sprawlers and swimmers in experiment 2.

Invertebrate taxa within the functional guilds in the drift also showed different responses to acid disturbances (Fig. 8, 9, and 10). Invertebrates in the collector/filterer functional group increased in the drift ($P < 0.05$) below acid disturbance at sites C (316% compared with reference area) and D (144%) in experiment 1 and at sites B (240%), C (483%), and D (140%) in experiment 3 relative to the reference area. The collector/gatherer functional group increased in the drift ($P < 0.05$) at sites C (260%) and D (188%) in experiment 1 and at site C (105%) in experiment 2, engulfers in the drift at site C (212%) in experiment 1 and at site D (85%) in experiment 3, and shredders at site C (86%) in experiment 1 and at sites B (204%) and C (64%) in experiment 3.

Invertebrates collected in the drift and partitioned into behavioural guilds likewise showed variable responses among the experiments. Significant increases in the drift ($P < 0.05$) of burrowers occurred at site D in experiments 1 (560% relative to site A) (Fig. 8) and 2 (421%) (Fig. 9). Clingers increased in the drift at sites C (305%) and D (145%) in experiment 1 (Fig. 8) and at sites B (240.7%), C (482%), and D (207%) in experiment 3 relative to the reference area (Fig. 10), swimmers at site D in experiments 1 (152%) and 2 (72%), and sprawlers at site D (122%) in experiment 3 only. Climbers appeared not to be affected whereas clingers responded most frequently by increased drifting (125–482% increase relative to the reference area for experiments 1, 2, and 3).

## Discussion

Mobilization of cations (total Al, IMA, Ca, and Mn) in the stream water during pH reduction in Lake 222 outflow was similar to the results of experiments conducted in south-central Ontario (Hall 1990) and the White Mountains of New Hampshire (Hall et al. 1980, 1987). Mobilized concentrations of the fraction potentially toxic to biota, IMA (Driscoll et al. 1980), however, were lower at Lake 222 outflow relative to Plastic Lake outflow (Dorset, Ontario) and Norris Brook (New Hampshire). The gradient in mobilization of IMA at different locations is thought to be, in part, attributed to the gradient in atmospheric $H^+$ loading

TABLE 4. Spearman's rank correlation between invertebrate drift communities (number·100 $m^{-3}$·$d^{-1}$) at sites A, B, C, and D and CA axes for acidification experiment 2. Asterisks indicate that absolute value $|r| > 0.40$ is significantly different from zero at the 5% level with the Bonferroni correction (Cooper 1968).

| | Life stage/ functional/ behavioural group[a] | Experiment 2 (pH 4.0) | | |
|---|---|---|---|---|
| | | CA1 | CA2 | CA3 |
| *Simulium* | L/CF/Cl | 0.726* | 0.369 | −0.093 |
| *Ectemnia* | L/CF/Cl | 0.809* | 0.419* | −0.048 |
| *Prosimulium* | L/CF/Cl | 0.289 | −0.454* | 0.108 |
| *Prosimulium* | P/CF/Cl | 0.173 | −0.432* | 0.418* |
| *Natarsia* | L/En/Sp | 0.062 | 0.569* | −0.388 |
| *Baetis* | N/CG/Sw | 0.479* | 0.107 | −0.464* |
| *Lepidostoma* | L/ShD/Sp | 0.042 | 0.272 | −0.504* |
| *Micropsectra* | L/CG/Cm | 0.238 | 0.356 | −0.501* |
| *Cricotopus* | L/ShD/Cl | 0.436* | 0.088 | −0.190 |
| *Hyalella* | ?/CG/Sw | −0.302 | −0.158 | −0.386 |
| *Thienemanniella* | L/CG/Sp | 0.528* | 0.364 | −0.284 |
| Orthocladiini | P/CG/Sp | 0.123 | −0.180 | 0.376 |
| *Ablabesmyia* | L/En/Sp | 0.010 | 0.200 | −0.338 |
| *Tanytarsus* | L/CG/Bu | 0.400* | 0.618* | −0.415* |
| *Eukiefferiella* | P/CG/Sp | −0.026 | −0.382 | −0.211 |
| Acarina | ?/CG/Sw | −0.075 | −0.106 | −0.198 |
| *Leptophlebia* | N/CG/Sw | −0.334 | 0.121 | −0.274 |
| *Procladius* | L/En/Sp | −0.138 | 0.171 | −0.679* |
| *Atherix* | L/En/Sp | 0.343 | −0.345 | 0.064 |
| *Corynoneura* | L/CG/Sp | 0.377 | −0.042 | −0.232 |
| *Brachycentrus* | L/CF/Cl | −0.367 | 0.179 | 0.259 |
| *Dixa* | L/CG/Sw | −0.020 | 0.150 | −0.111 |
| *Nectopsyche* | L/ShD/Cm | −0.375 | −0.048 | 0.034 |
| Tanytarsini | P/CG/Bu | 0.303 | −0.275 | −0.138 |
| *Eukiefferiella* | L/CG/Bu | −0.127 | 0.547* | −0.443* |
| Eigenvalue | | 0.1235 | 0.0761 | 0.545 |
| Percent | | 31.45 | 19.39 | 13.88 |
| Cumulative | | 31.45 | 50.84 | 64.72 |

[a] See Table 2 for definitions.

and differential weathering of minerals (LaZerte and Dillon 1984). Strong acid deposition averaged 8–10 meq·$m^{-2}$·$yr^{-1}$ in northwestern Ontario (ELA, Schindler et al. 1976), 70–100 in the Muskoka–Haliburton highlands (near Dorset) in south-central Ontario (Dillon et al. 1980), and 120 meq·$m^{-2}$·$yr^{-1}$ at Hubbard Brook Experiment Forest, New Hampshire (Likens et al. 1977). During experimental acidification at ELA, biotic communities were exposed to low levels of potentially toxic IMA relative to experiments in other regions, and thus, the responses of biota in Lake 222 outflow are more likely correlated with the increased $H^+$ concentration than with increased IMA.

Short-term pH disturbances at ELA produced significant changes in lake outflow benthic communities. However, the large reductions in benthic densities at Lake 222 outflow occurred at higher pH (5.0) during shorter exposure (4 d) and without a concomitant increase in Al (total Al and IMA) compared with pH disturbance experiments in Plastic Lake outflow that were conducted for 8 d and at pH depressions 4.8 and 4.2. In contrast, reductions in benthic densities at pH 5.0 have not been reported in other short-term experimental studies (Ormerod et al. 1987; Hopkins et al. 1989). As $H^+$ intensity was increased from 4.5 to 4.0 at Lake 222 outflow, additional species responded, some taxa similar to those of Plastic Lake outflow and some that had not been reported previously. Overall, at pH 4.0, more species at ELA responded than in Plastic Lake outflow (Hall 1990).

Diversity values contrasted sharply in areas of low and high acid deposition. In Lake 222 outflow, generic diversity was reduced at pH 4.0 relative to 6.9 whereas no changes in diversity were evident below similar pH disturbances (4.0 relative to 5.6) in Plastic Lake outflow (Hall 1990). However, generic richness was reduced at both of these locations. The only comparable study in which an episodic pH reduction to near 4.0 produced reductions in diversity was conducted in a hardwater stream (Herricks and Cairns 1974). The mechanisms responsible for reductions in diversity at ELA but not at Dorset may be related to prior replacement of acid-sensitive species by acid-tolerant ones at the latter location (Hall et al. 1980; Hall and Ide 1987), physiological acclimation of individuals in areas of high acid deposition, evolution of resistance (Swarts et al. 1978) to stresses that alter osmoregulatory processes in individuals, or stage of life cycle when acid stresses occur (Hall 1990). Another mechanism that may be involved is the physical force brought about by fluctuating water flow. Recently, investigators reported that unpredictable fluctuation in discharge may be more of an organizing force in structuring invertebrate

TABLE 5. Spearman's rank correlation between invertebrate drift communities (number·100 $m^{-3}$·$d^{-1}$) at sites A, B, C, and D and CA axes for acidification experiment 3. Asterisks indicate that absolute value $|r| > 0.40$ is significantly different from zero at the 5% level with the Bonferroni correction (Cooper 1968).

| | Life stage/ functional/ behavioural group[a] | Experiment 3 (pH 5.0) | | |
|---|---|---|---|---|
| | | CA1 | CA2 | CA3 |
| *Simulium* | L/CF/Cl | 0.945* | −0.279 | −0.388 |
| *Ectemnia* | L/CF/Cl | 0.626* | −0.317 | −0.307 |
| *Sphaerium* | ?/CF/Cl | 0.675* | −0.739* | −0.504* |
| *Hyalella* | ?/CG/Sw | 0.755* | −0.517* | −0.496* |
| *Prosimulium* | L/CF/Cl | −0.566* | −0.358 | −0.209 |
| Acarina | ?/CG/Sw | 0.583* | −0.288 | −0.566* |
| *Lepidostoma* | L/ShD/Sp | 0.045 | −0.240 | 0.050 |
| *Micropsectra* | L/CG/Cm | 0.017 | 0.017 | 0.315 |
| *Natarsia* | L/En/Sp | 0.294 | 0.060 | 0.038 |
| *Leptophlebia* | N/CG/Sw | 0.632* | −0.350 | −0.051 |
| *Cricotopus* | L/ShD/Cl | −0.037 | −0.077 | −0.204 |
| *Procladius* | L/En/Sp | 0.406* | −0.589* | −0.028 |
| *Prosimulium* | P/CF/Cl | 0.202 | −0.367 | −0.332 |
| *Ablabesmyia* | L/En/Sp | 0.142 | −0.053 | −0.125 |
| Harpacticoida | | 0.133 | −0.314 | −0.446* |
| *Tanytarsus* | L/CG/Bu | 0.420* | −0.141 | 0.249 |
| *Baetis* | N/CG/Sw | 0.468* | −0.134 | 0.129 |
| *Eukiefferiella* | P/CG/Sp | 0.359 | −0.173 | 0.128 |
| Pentaneurini | P/En/Sp | 0.468* | −0.324 | −0.052 |
| Orthocladiini | P/CG/Sp | 0.348 | −0.542* | 0.025 |
| Cladocera | ?/CG/Sw | 0.147 | −0.531* | −0.373 |
| *Macronema* | L/ShD/Cm | 0.612* | −0.671* | −0.437* |
| Chironomini | P/CG/Bu | 0.512* | −0.309 | −0.103 |
| *Hydroscapha* | L/Sc/Cl | 0.494* | −0.198 | −0.084 |
| *Dixa* | L/CG/Sw | −0.435* | 0.252 | 0.425* |
| Eigenvalue | | 0.2084 | 0.1339 | 0.0515 |
| Percent | | 36.51 | 23.47 | 9.03 |
| Cumulative | | 36.51 | 59.97 | 69.00 |

[a] See Table 2 for definitions.

communities than pH and associated parameters (Giberson and Hall 1988; Resh et al. 1988; Hall 1990). However, results in this study show no strong correlation with discharge. Similar conclusions were reached following a rainstorm in which discharge increased rapidly in an experimentally acidified stream in Wales (Weatherley et al. 1988).

The mayfly *Baetis* responded by significant increases in the drift and decreases in benthic density during pH reductions. Nymphs and eggs of this genus are sensitive to pH reductions ≤5.7 in the laboratory (Engblom and Lingdell 1984; Raddum and Fjellheim 1984; Rowe et al. 1988). In field experiments, *Baetis* individuals exhibited sensitivity when transplanted either to acidic water or to stream water that was experimentally acidified (Minshall and Minshall 1978; Allard and Moreau 1987; Ormerod et al. 1987; Hopkins et al. 1989). Other investigators found mortalities of up to 25–50% for *Baetis* when whole-stream reaches (Ormerod et al. 1987) or channels (Hopkins et al. 1989) had been acidified to pH 4.5 or 5.0 for periods ranging from 5 to 24 h. Furthermore, *Baetis* was not collected in surveys in acidic streams in North America (Fiance 1977; Kimmel et al. 1985; Mackay and Kersey 1985; Peterson et al. 1985; Simpson et al. 1985; Giberson and Mackay 1990; Smith et al. 1990; Peterson and Van Eeckhaute 1992; Rosemond et al. 1992) and Europe (Minshall and Kuehne 1969; Sutcliffe and Carrick 1973; Matthias 1973; Otto and Svenson 1983; Raddum and Fjellheim 1984). Since the effects of acidic water on *Baetis* appear to be ubiquitous, this genus is a good candidate as an early-warning indicator for onset of acidification.

The increased drift during pH disturbances could be due to active, passive, or toxic responses of individual taxa or a combination of these (Hall et al. 1980, 1985; Allard and Moreau 1984, 1987; Raddum and Fjellheim 1984; Griffiths 1987, 1992; Hopkins et al. 1989). Raddum and Fjellheim (1984) reported that initially, *Baetis* actively avoided experimental $H^+$ stress (pH ≤ 5.5) in laboratory and field experiments, but after 24 h, nymphs reduced their activity (passive drift due to sublethal stress) and then eventually succumbed to acid stress. Similarly, reduced activity with onset of acidification has been reported in zooplankton (Havas 1985) and fish (Muniz and Leivestad 1980).

Reduced drift activity in the blackfly *Prosimulium* in Lake 222 outflow experiments differs from that in experimental studies conducted at Norris Brook, New Hampshire, in which the larvae of this genus repeatedly increased in the drift during reduced pH disturbances (Hall et al. 1980, 1987). This differential response to elevated acidity may be related to pH sensitivity of different life stages (eggs, larvae, and pupae; Hall et al. 1988; Chmielewski and Hall 1992) and/or different species responses within the *Prosimulium* complex

TABLE 6. Spearman's rank correlations between the invertebrate drift community based on the CA ordination and abiotic variables for three pH disturbance experiments in the ouflow of Lake 222. Asterisks represent significant correlation ($P < 0.05$).

| Chemical and physical parameters | Experiment 1 (pH 4.5) | | | Experiment 2 (pH 4.0) | | | Experiment 3 (pH 5.0) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Axis 1 | Axis 2 | Axis 3 | Axis 1 | Axis 2 | Axis 3 | Axis 1 | Axis 2 | Axis 3 |
| pH | 0.45* | −0.63* | −0.21 | −0.61* | −0.40* | 0.25 | −0.45* | −0.02 | −0.07 |
| Discharge | 0.02 | −0.29 | −0.01 | −0.04 | 0.06 | 0.03 | 0.07 | −0.29 | −0.10 |
| DOC | −0.51* | 0.54* | 0.18 | 0.46* | 0.36 | −0.19 | −0.07 | −0.07 | 0.19 |
| Ca | −0.46* | 0.45* | 0.08 | 0.36 | 0.03 | −0.02 | 0.42* | 0.06 | 0.07 |
| Al | −0.34 | 0.37 | 0.35 | 0.44* | 0.25 | −0.59* | 0.30 | −0.14 | 0.14 |
| IMA | −0.43* | 0.64* | 0.27 | 0.42* | 0.45* | −0.29 | −0.04 | 0.04 | 0.18 |
| Fe | −0.11 | 0.34 | 0.39 | −0.13 | −0.63* | 0.20 | −0.31 | −0.11 | 0.02 |
| Mn | −0.20 | 0.24 | 0.09 | 0.12 | 0.06 | 0.17 | −0.05 | −0.12 | 0.18 |
| Conductivity | −0.33 | 0.51* | 0.52* | 0.58* | 0.36 | −0.19 | 0.47* | 0.02 | 0.02 |

(Hall et al. 1988). Chmielewski and Hall (1992) reported that pupae of simuliid taxa became malformed during short-term pH reductions, the degree of abnormalities being related to $H^+$ intensity and the time of the pulse in relation to the stage of transformation (i.e., early or later completion of the pupal stage). At the time of the ELA study, pupation of *Prosimulium* individuals was well advanced, corresponding to the observed decrease in drift of larvae. If pupae are particularly sensitive, as shown by Chmielewski and Hall (1992), then the observed decrease in numbers of this genus would be expected and explained as pupal mortality. Most of the other *Simulium* spp. were in the larval stage, however, and drifted downstream with increased acidity.

The role that behaviour plays in the response of benthic invertebrates to pH reduction is also evident in this study. For both drift and density data, species (or taxa) that responded either by increases or decreases in density during pH disturbances were those predominately in swimmer, climber, clinger, or sprawler behavioural guilds. These individuals are located generally above sediments within the water column where direct exposure to increased acidity and metal occurs. Collins et al. (1981) and Dermott et al. (1986) also concluded that the epifauna was altered in acidic lakes but not the infauna. However, burrowers are not completely protected, as indicated by the genus *Tanytarsus*. In the second experiment at pH 4.0, *Tanytarsus* significantly increased in drift and decreased in the benthos in the acidified section relative to reference areas, but no change in drift or benthic density was evident for this genus in experiment 3. In contrast, a significant increase in density occurred in the acidified area relative to the reference location in experiment 1. A variable response of *Tanytarsus* to $H^+$ stress was also observed by Hall (1990) in another lake outflow stream. Since *Tanytarsus* has been shown to be moderately (Allard and Moreau 1987; Griffiths 1992) to highly sensitive (Bell 1970) to pH reductions, variability in response to elevated acidity in the field may be modified by the influence of habitat. More specifically, individuals within the genus *Tanytarsus* may be able to survive acid pulses longer in habitats with increased amounts of organic debris than in areas with more sandy habitats (Hall 1990) because organic debris can neutralize a greater quantity of $H^+$ than sandy sediments (Oliver and Kelso 1983).

Excluding filter-feeders, similar genera are found in the outflow and in the littoral areas of the lake upstream (R. Reid, Dorset Research Centre, personal communication) as well as in shallow areas of lakes in other localities (Uutala 1981; Dermott 1985; Dermott et al. 1986; Griffiths and Keller 1992). Some taxa (*Procladius* and *Microtendipes*) that appeared tolerant in lake outflow acidification experiments in the present study and others (Hall 1990) were reported to be abundant in littoral areas of acidic lakes (Bradt and Berg 1987; Griffiths and Keller 1992; Stephenson et al. 1994), while other taxa (e.g., *Hyalella*) that have disappeared from acidic lakes (Stephenson and Mackie 1986) showed strong sensitivity by drifting in the present experimental stream acidification (Table 5). An added benefit, then, from this study is that experimental acidification in the lake outflow provided data on acidic sensitivities and tolerances of lentic genera with less effort than experimentally acidifying or liming the whole lake.

In conclusion, short-term pH depressions in the stream water reduced invertebrate diversity, increased drift and decreased benthic density in Lake 222 outflow. More striking, however, was the fact that reductions in numbers of biota on the stream bottom occurred at higher pH levels (5.0) without a rise in Al concentration and more quickly than at Plastic Lake outflow, supporting my original hypothesis that organisms in more pristine habitats are more sensitive to acidification. This differential response of invertebrates to experimentally elevated $H^+$ concentration correlated with the amount of acidic deposition in these regions, less deposition occurring at ELA, Ontario, relative to Dorset, Ontario, and the Hubbard Brook Experimental Forest, New Hampshire. More recently, on the west coast of British Columbia, Bernard et al. (1990) reported that invertebrates in a chemically dilute stream that was experimentally acidified from 6.9 to 5.9 behaviourally avoided this stress. The pH levels that produced these avoidance responses were lower than those of any other studies in North America, further corroborating the results of my experiment.

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# Oocyte Recruitment in First-Time Spawning Atlantic Cod (*Gadus morhua*) in Relation to Feeding Regime

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Kjesbu, O.S., and J.C. Holm. 1994. Oocyte recruitment in first-time spawning Atlantic cod (*Gadus morhua*) in relation to feeding regime. Can. J. Fish. Aquat. Sci. 51: 1893–1898.

Adolescent (10-mo-old, ≈165 g) Atlantic cod (*Gadus morhua*) hatched and reared in the laboratory were transferred to three different feeding regimes (groups) for a full year, high–high (HH), low–high (LH), or high–low (HL), switching the ration size after 5 mo in July (i.e., before vitellogenesis). Subsequent analysis of fecundity before the first spawning season revealed that adolescent body weight was most important for fecundity followed by growth rate in the autumn, while growth rate in the spring contributed insignificantly. There existed a significant positive correlation between individual, starting (adolescent), and final body weight. All groups were characterized by high individual variability in both fecundity and oocyte development, suggesting strong genetic influences. The groups showed different final mean body weights (HH: 1265 g, LH: 1199 g, and HL: 886 g) but, unexpectedly, the same mean relative fecundities (number of oocytes per gram of ovary-free fish) (≈1200). The study on oocyte sizes provided evidence that HH fish will commence spawning earliest in the year followed by LH fish and then HL fish.

Des morues branches (*Gadus morhua*) de 10 mo (≈ 165 g) écloses et élevées au laboratoire ont été soumises à trois régimes alimentaires (groupes) différents pendant une année complète dans laquelle la ration a été changée après 5 mo en juillet (c.-a-d. avant la vitellogenèse), soit les régimes à ration élevée–élevée (EE), faible–élevée (FE) ou élevée–faible (EF). Une analyse subséquente de la fécondité avant la première saison de frai a révélé que le poids corporel des jeunes morues était le plus important pour la fécondité suivi du taux de croissance à l'automne, alors que le taux de croissance au printemps avait peu d'effet. On a observé une corrélation positive significative entre le poids initial (à 10 mo) et le poids final des individus. Tous les groupes étaient caractérisés par une variabilité individuelle élevée en ce qui a trait à la fécondité et au développement de l'ovocyte, ce qui indique de fortes influences génétiques. Le poids corporel moyen final était différent chez les trois groupes (EE : 1 265 g; FE : 1 199 g et EF : 886 g) mais, contre toute attente, la fécondité relative moyenne (nombre d'ovocytes par g d'ovaire-poisson frais) était la même (≈ 1 200). L'étude de la taille des ovocytes a indiqué que les poissons soumis au régime EE commencent à frayer plus tôt au cours de l'année, suivis des poissons soumis au régime FE, puis des poissons soumis au régime EF.

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This paper examines the effects of experimentally varied feeding regimes on fecundity in 2-yr-old recruit (first-time) spawning Atlantic cod (*Gadus morhua*) using specimens hatched and raised in the laboratory. Bagenal (1978) defined fecundity as the number of vitellogenic oocytes present in the ovary shortly before spawning. Recently, Rijnsdorp et al. (1991) showed significant annual variation in relative fecundity (number of vitellogenic oocytes per gram body weight) in cod from the south and central North Sea. Relative fecundity increased by about 20% from 1970–71 to 1987–88. Concurrently, spawning stock biomass decreased by about two thirds. Fecundity may increase with food availability (Lett et al. 1975). Rijnsdorp et al. (1991) concluded that a better understanding of recruitment variability requires more knowledge of reproductive biology of recruit spawners. Beacham (1983) and Jørgensen (1990) noticed long-term reductions in age at maturity in the Northwest and Northeast Atlantic cod stocks, respectively. No such trend has been documented in the North Sea, but about 20% of males and about 10% of females mature sexually as 2-yr-olds (Rijnsdorp et al. 1991). Similar data exist for cod on the Scotian Shelf (Beacham 1983). The phenomenon of early sexual maturity in Atlantic cod is of interest to aquaculture: early gonadal growth means less somatic growth and reduced economic production. In Norwegian Coastal cod, 100% of the 2-yr-old captive females can mature sexually (Lehmann et al. 1991). The lower threshold of sexual maturity in Atlantic cod is about 30 cm (Rijnsdorp et al. 1991; Trippel and Neilson 1992).

The effect of food supply on fecundity in recruit Atlantic cod has attracted little attention. Waiwood (1982) demonstrated the importance of growth rate to fecundity but pooled recruit and repeat spawners. Kjesbu et al. (1991) found a significant, positive relationship between fecundity and condition factor in repeat spawners. Holdway and Beamish (1985) studied the dynamics of oocyte growth using

TABLE 1. Overview of experimental design. Each regime was kept in a separate sea cage. $n$ = number of fish (both sexes) in each cage; cage volume = total volume of seawater; period 1: February–July; period 2: July–January.

| Regime | $n$ | Cage volume ($m^3$) | Feeding frequency |  |
|---|---|---|---|---|
|  |  |  | Period 1 | Period 2 |
| HH | 500 | 100 | 6 d·wk$^{-1}$ | 6 d·wk$^{-1}$ |
| LH | 500 | 100 | 6 d·wk$^{-1}$ | 6 d·wk$^{-1}$ |
| HL | 200 | 100 | 6 d·wk$^{-1}$ | 6 d fed, 8 d off |

histological techniques, but gave no estimates of fecundity. Like Waiwood (1982), they transported wild cod to the laboratory to be fed different rations. In contrast, Kjesbu et al. (1991) used hatchery-reared cod in their experiment. Fish with similar rearing backgrounds were used in the present investigation. Our objectives were to (1) quantify effects of various feeding regimes, established for a full year, on the subsequent fecundity in 2-yr-old cod (i.e., in fish sampled shortly before first-time spawning), (2) examine if there was any period in the year in which the fecundity was particularly sensitive to food variations, and (3) demonstrate any effects of regimes on oocyte growth and thereby spawning time.

## Materials and Methods

### Fish and Study Site

The fish were reared artificially at the Austevoll Aquaculture Research Station, Norway, in 1988 according to methods of Holm and Andersen (1989) and Naas (1990). The broodstock, 5-yr-old Norwegian Coastal cod, included 264 females and 148 males mating randomly. The experiment started in February 1989, when the adolescent fish were 10 mo old, and terminated in January 1990, just prior to the first spawning season. The initial study population, February 1989, comprised 7709 specimens, all individually tagged (Floy tag) and measured. Of these, 1212 were diagnosed from a subgroup, the following year, as females using ultrasonography (Mattson 1991; Karlsen 1992).

### Feeding Regime and Physical Parameters

Three feeding regimes (groups) were defined and designated by ration size, high or low, as high–high (HH), low–high (LH), and high–low (HL). Each group was kept in a single, separate sea cage placed side by side with the other two identical ones at the Austevoll Aquaculture Research Station (Table 1). In July, after 5 mo, the ration size was switched, i.e., prior to the onset of vitellogenesis in Setember–November (Kjesbu et al. 1991). Fish on high ration were fed to satiation. Low ration was half of the high ration (based on a per gram body weight basis). No low–low regime was established, as this would (1) produce fish of no interest in commercial aquaculture and (2) initiate resorption of vitellogenic oocytes (atresia) complicating fecundity estimation (Kjesbu et al. 1991). Ration size for each group was adjusted in relation to its total biomass i.e., summed weight of all individuals in the cage recorded at the start, middle, and end of the experiment, the water temperature, and the expected daily growth using equations established by Jobling (1988).

All fish were given dry feed pellets by hand. Fish on high ration were fed 6 d·wk$^{-1}$. This feeding frequency was also used for the fish on low ration during February–July (period 1) to reduce cannibalism (Folkvord 1991). During the second part of the regimes, July–January, the fish on low ration were fed every other week (i.e., 6 d fed, 8 d fasted), as the bigger fish were not cannibalistic. Feeding large meals to fish on low ration resulted in a more even distribution of food among fish than feeding smaller meals more frequently would have done. Stocking density was low, reaching maximum 5 kg fish·m$^3$ seawater$^{-1}$ at the end of the experiment. The feed (Skretting Marine, T. Skretting A/S, Norway) had an available energy content of 13.64 kJ·g wet weight$^{-1}$ (water content 10.7%) with 56.5, 15.8, and 27.7% protein, fat, and carbohydrate, respectively. Water temperature (at 2 m depth) varied from about 5 and 7°C at the start and end of the experiment, respectively, to a maximum of about 14°C in June–September. Salinity ranged 25–32‰ but was typically around 30‰.

### Fish Measurements

Total length (TL) (±1 mm) and wet weight ($W$) (±5.0 g) were measured at the start (February), middle (July), and end of the experiment (January). We used metomidate as an anaesthetic (Mattson and Riple 1989). Fulton's condition factor ($K$) was calculated as

$$(1)\quad K = \frac{W}{\mathrm{TL}^3} \cdot 100.$$

Specific growth rate ($G$) was defined as

$$(2)\quad G = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \cdot 100$$

where $t$ represents the time in days. $G$ was calculated for both periods 1 and 2 and symbolized by $G_1$ and $G_2$. However, to compare the two rates directly, the term $G_{2,\mathrm{adj}}$ was introduced to exclude the effect of the increase in ovary weight caused by vitellogenesis during period 2. It was assumed that the stroma and previtellogenic oocytes contributed 5% to the ovary weight (Buzeta and Waiwood 1982: Atlantic cod; Hay and Brett 1988: Pacific herring (*Clupea pallasi*)); 95% of the final wet ovary weight was subtracted from the final wet body weight.

### Sampling Design

At the end of the experiment, about 40 females, selected by total length to give the best possible overlap in length range (32–54 cm) among groups, were killed in each group. Thus, we intended to use ANCOVA when contrasting the groups for fecundity. However, multiple regression analysis demonstrated that there was a significant, positive influence of starting weight (i.e., adolescent body weight) on the subsequent fecundity (see below). Comparing the mean adolescent body weight of each group with the corresponding population mean (162 g (SD = 71), $n$ = 1212), females included in the HH and HL groups were found to be significantly below (122 g (SD = 54)) and above (205 g (SD = 66)) the population mean ($P < 0.0005$, one-tailed $t$-test), respectively, while the LH females were of average weight (163 g (SD = 73)) ($P > 0.45$). Consequently, our pilot fecundity tests among groups became biased.

As a result, for the comparison of fecundity, further selective sampling was required to obtain samples among the three groups with similar starting weights (note that all fish were tagged individually); plots showed that the sizes among groups overlapped. We selected triplets of fish, one from each group, with similar adolescent weights (±10 g) within a standardized size-class (75–250 g). As noted, this procedure reduced the number of females in each group but produced groups with similar starting weights ($P > 0.90$, ANOVA).

### Processing of Organs and Fecundity Estimation

Fecundity was defined in this study as the total number of vitellogenic oocytes in the prespawning ovary (Bagenal 1978). All ovaries were therefore screened to exclude spawning fish; a partially spawned fish would confound the analysis. Four females were excluded due to the presence of hyaline oocytes in ovaries or <6000 vitellogenic oocytes·g wet ovary$^{-1}$, both characters indicating commencement of spawning (Kjesbu et al. 1990).

The ovary, liver, and viscera were removed and weighed (±0.1 g). Ovary and liver weights were divided by the whole-body weight to give the gonadosomatic index (GSI) and hepatosomatic index (HSI) (both expressed as percentages), respectively. The ovaries were put on ice immediately after weighing, transported to the Institute of Marine Research in Bergen, and frozen at −18°C. The ovary was reweighed shortly after thawing. All ovarian subsamples were subsequently weighed (±1 mg) and placed in a solution of sucrose (165 g·L distilled water$^{-1}$) and 2% formaldehyde. This solution was 790 mosmol (Vapor Pressure Osmometer, Wescor, Inc.), approximately double the osmolality in the ovoplasm (Mangor-Jensen 1987). The high concentration of sucrose raised the refractive index increasing the contrast, under the binocular microscope, between the vitellogenic oocytes, which darkened in colour, and the transparent petri dish. Furthermore, the high viscosity/osmolality probably prevented oocytes from disintegrating, as this was not observed in the subsamples after gentle shaking to separate the oocytes.

The fecundity was determined gravimetrically: the number of vitellogenic oocytes in the subsample was multiplied by the whole-ovary (thawed) weight divided by the subsample weight. The Atlantic cod has a determinate fecundity; there is no new recruitment of oocytes to the stock of vitellogenic oocytes during spawning (West 1970; Kjesbu et al. 1990). Relative fecundity was calculated as fecundity divided by ovary-free whole-body weight. The present 109 ovaries were examined for fecundity by collecting one subsample from each fish and counting about 1000 oocytes. A pilot test confirmed that oocyte size did not vary among different locations in the ovary, and only 0.2% of overall variation was attributable to variation between replicate samples (nested ANOVA).

### Vitellogenic Oocyte Size

Because ovaries had been frozen and placed in a strongly hyperosmotic fluid, oocyte diameter was not a reliable measure. The number of oocytes per gram wet ovary (NG) was, however, not affected and was used to estimate the mean fresh vitellogenic oocyte diameter ($D$), assuming the oocytes were spherical and had a specific gravity of 1.0464 (tests on three ovaries). A similar technique was used by Kucera and Kennedy (1977) to calculate fecundity from data on ovary volume and mean oocyte volume. Stroma and

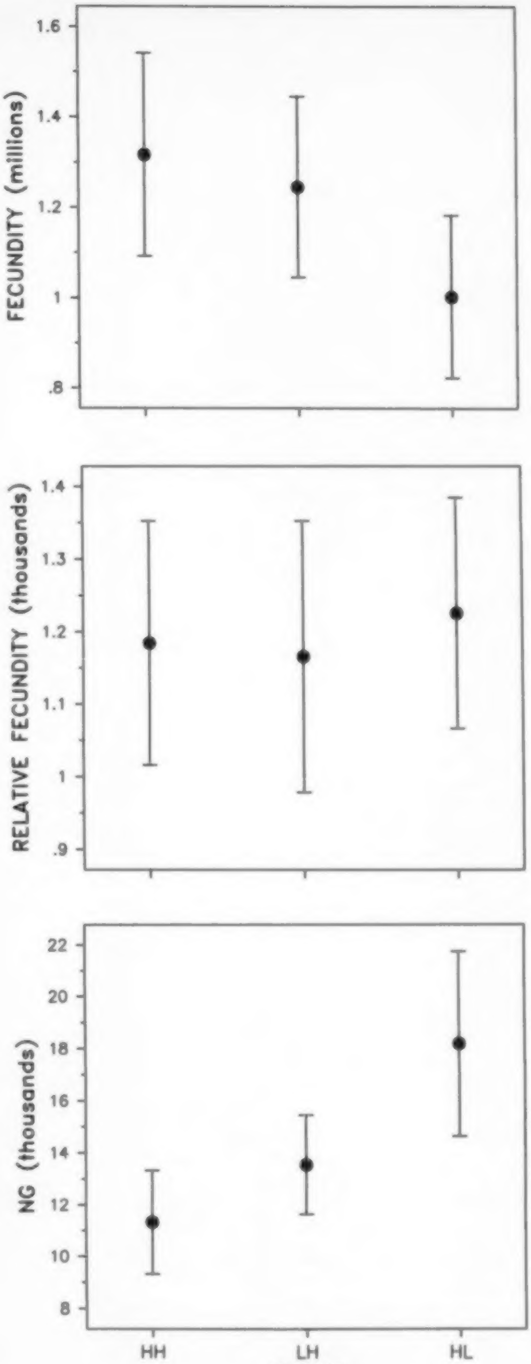

FIG. 1. Fecundity (top panel), relative fecundity (middle panel), and number of vitellogenic oocytes per gram wet ovary (NG) (bottom panel) with 95% confidence limits in the three feeding regimes HH, LH, and HL at termination of the experiment (21-mo-old fish). NG is inversely related to oocyte diameter (stage of maturity). Each regime is represented by 19 females with similar starting weights.

TABLE 2. Summary of fish data for females selected as adolescents for similar starting weights among regimes (groups) HH, LH, and HL. Mean body weight ($W$) and mean specific growth rate ($G$) throughout the experimental period in each of the three groups are given. The resulting fish at termination of the experiment are described by the mean Fulton's condition factor ($K$), gonadosomatic index (GSI), and hepatosomatic index (HSI). For all parameters, SD is given in parentheses. $W_{adw}$ = adolescent whole-body weight (10-mo-old fish); $W_{July}$ = whole-body weight halfway through the experiment; $W_{Jan}$ = whole-body weight at termination of the experiment; $G_1$ = specific growth rate in the first half of the experiment; $G_{2,adj}$ = specific growth rate in the second half of the experiment but excluding the increase in ovary weight during vitellogenesis; $n$ = number of females (the same females were followed throughout the year).

| Regime | $n$ | $W_{adw}$ (g) Feb. 1989 | $W_{July}$ (g) July 1989 | $W_{Jan}$ (g) Jan. 1990 | $G_1$ Feb.–July 1989 | $G_{2,adj}$ July 1989 – Jan. 1990 | $K$ Jan. 1990 | GSI (%) Jan. 1990 | HSI (%) Jan. 1990 |
|---|---|---|---|---|---|---|---|---|---|
| HH | 19 | 164 (46) | 469 (127) | 1265 (332) | 0.691 (0.072) | 0.496 (0.062) | 1.36 (0.11) | 10.0 (3.0) | 9.5 (0.9) |
| LH | 19 | 163 (44) | 381 (79)[a] | 1199 (309) | 0.566 (0.097)[a] | 0.592 (0.064)[a] | 1.28 (0.12) | 8.1 (2.4) | 9.5 (0.8) |
| HL | 19 | 165 (44) | 494 (121)[a] | 886 (217) | 0.713 (0.051)[a] | 0.289 (0.056)[a] | 1.17 (0.10) | 6.9 (2.3) | 7.7 (0.8) |

[a] One data point missing.

previtellogenic oocytes were assumed to contribute 5% totally to the ovary weight (see above). The established formula relating NG and $D$ was checked against data in Kjesbu et al. (1990) showing an underestimation in oocyte diameter by about 18 μm, the final formula being

(3) $D = 18 + 1.201 \times 10^4 \cdot \mathrm{NG}^{-1/3}$ $(6750 < \mathrm{NG} < 21\ 500)$.

## Results

### Fecundity

Fecundity among the three groups was significantly different ($0.01 < P < 0.05$, Kruskal–Wallis test) (Fig. 1). This test was selected, as one of the groups (HL) was nonnormally distributed ($P \approx 0.005$, Kolmogorov–Smirnov (Lilliefors) test). The effect of feeding regime was due to the HL group, as there was no significant difference between the HH and LH groups ($P > 0.60$, Student $t$-test). The groups were not significantly different in terms of relative fecundity ($P > 0.75$, ANOVA) (Fig. 1) but differed in their final body weights ($P < 0.001$, ANOVA) (Table 2), again due to the HL fish, as the HH and LH fish were not significantly different ($P > 0.50$, Student $t$-test). All fish tested were within the same standardized weight-class, initially (Table 2).

Multiple regression analysis was applied to test the influence of starting weight (adolescent body weight) ($W_{adw}$) in addition to feeding regime, using the specific growth rates during spring ($G_1$) and autumn ($G_{2,adj}$), on the subsequent fecundity in individual fish. The coefficient of multiple determination ($R^2$) was found to be 0.405 ($P < 0.001$, $n$ = 105). The standardized coefficients (Sokal and Rohlf 1981) for $W_{adw}$, $G_1$, and $G_{2,adj}$ were 0.803, 0.151, and 0.363, respectively; $W_{adw}$ contributed 5.3 times more to fecundity than $G_1$ and 2.2 times more than $G_{2,adj}$. The subsequent $F$-tests showed, however, that the contribution of $G_1$ was insignificant ($P \approx 0.13$). Omitting $G_1$ from the regression, $W_{adw}$ contributed 2.3 times more than $G_{2,adj}$ (both contributions with $P < 0.001$). Finally, fecundity was regressed on whole-body weight at the midpoint of the experiment, $W_{July}$, and on $G_{2,adj}$. This showed that the contribution of $W_{July}$ relative to $G_{2,adj}$ was 1.6, both contributing significantly ($P < 0.001$), $R^2$ being 0.393 ($P < 0.001$).

The influence of starting weight on fecundity was examined more closely in two ways, studying each group separately: firstly by regressing relative fecundity on starting weight finding no significant relationships ($P > 0.30$), and secondly by regressing final weight on starting weight uncovering significant linear regressions ($P < 0.001$); $r^2$ = 0.727, 0.786, and 0.799 in the HH, LH, and HL groups, respectively (Fig. 2). Similar results were obtained by including fish from the standardized size-class only.

### Vitellogenic Oocyte Size

NG was calculated for each specimen in the standardized size-class. Each feeding regime was studied separately to test for effects of final fish weight on NG. No such significant correlation existed ($P \gtrsim 0.20$). The mean NG was estimated to 11 300 in the HH group, 13 500 in the LH group, and 18 200 in the HL group (Fig. 1). The observed differences were tested by the Games and Howell test to account for heterogeneous variances ($P \approx 0.01$, Barlett test) but normal distributions ($P > 0.60$, Kolmogorov–Smirnov (Lilliefors) test). There was a significant difference between the HH and HL groups ($P = 0.05$) but not between HH and LH ($P >> 0.05$).

By use of formula (3), the corresponding mean fresh vitellogenic oocyte diameters were found to be 553, 522, and 475 μm. Thus, the mean oocyte volume was 58 and 33% higher in the HH and LH groups than in the HL group, respectively. Application of a recent developed method for forecasting the time to start of spawning from oocyte diameters (Kjesbu 1994) showed 24, 27, and 33 d for the HH, LH, and HL groups, respectively. Thus, relative to the HH fish, mean spawning time was delayed by 3 d in the LH fish and 9 d in the HL fish.

## Discussion

The mean relative fecundity was similar in the HH, LH, and HL regimes. Thus, differences in mean fecundity were the result of differences in growth rates which produced fish of different final size. Growth rates were within the range recorded for cultured cod under similar feeding and environmental conditions (Lehmann et al. 1991). The original sampling design masked real differences in mean fecundity. This bias was, however, adjusted in our analysis by using a standardized initial size-class (based on tagged individuals) in light of the finding that the starting (adolescent) body weight was most important for the subsequent fecundity. Starting and final weights were closely, positively correlated; adolescent body weight expressed the capacity of an individual for further growth. Contrasting recruit and repeat spawning captive Atlantic cod, the latter (Kjesbu et al. 1991)

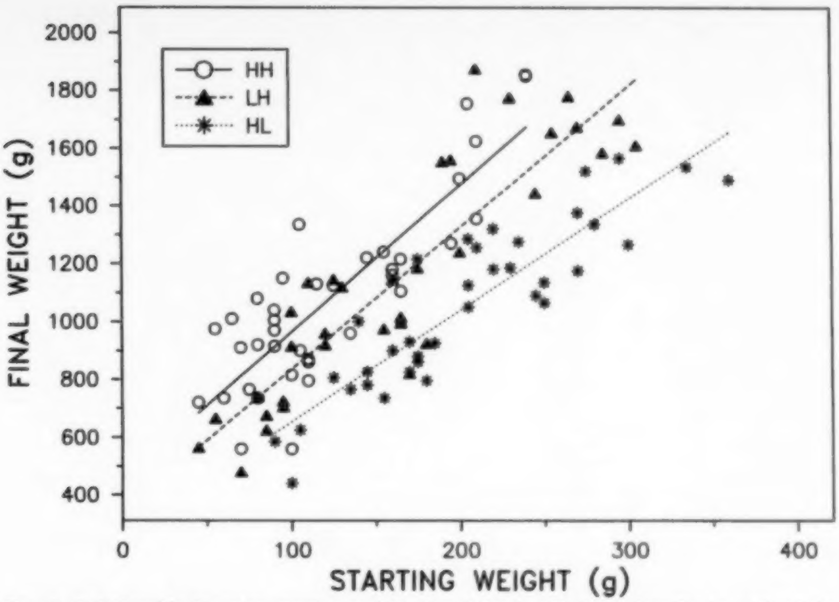

FIG. 2. Relationship between starting and final weights in all single females in each of the three feeding regimes. Lines were established by least squares regression.

appear to be more sensitive to variations in feeding regime during vitellogenesis. Repeat spawners experience severe depletion of body reserves following spawning (Smith et al. 1990; Kjesbu et al. 1991) and are therefore more sensitive on subsequent feeding regimes. Further, repeat spawners typically show higher relative fecundities (Kjesbu et al. 1991) enhancing this dependence.

With respect to production of new oocytes in recruit spawners, the specific growth rate in the first half of the year has little effect relative to the specific growth rate in the last half of the year, which is important. Cod from the Bay of Fundy, Canada, were held in captivity from June to March (Waiwood 1982). Waiwood (1982) defined the most important period for oocyte production to be from June to September but did not separate the recruit and repeat spawners. There were also indications that the specific growth rate for immature fish in the same period was positively correlated with the onset of maturation. To date, little is known about the detailed aspects of oocyte production, the main reason being that traditional histological methods will not work well in this respect (Emerson et al. 1990).

Shirokova (1977) stressed that there is "considerable individual variation" in the time of occurrence of the different developmental phases of previtellogenic oocytes in juvenile Baltic cod. In a similar manner, the present study on the vitellogenic oocytes just prior to the first spawning season in Norwegian coastal cod shows a large range in the mean diameter within each feeding regime. The observation of bigger oocytes in the better-fed fish can be interpreted in two basically different ways: better-fed fish produce bigger eggs or the fish are in a more advanced stage of maturity. A combination of the two is a third possibility. In Atlantic cod, a multiple-batch spawner, it is known that the egg size is determined during the 2- to 3-d interval between batches (Kjesbu et al. 1991). Also, it has been the practice in the laboratory to predict the time of start of spawning of each individual female cod by diameter measurements on vitellogenic oocytes sampled by catheterization some months prior to spawning (Kjesbu 1994). Thus, there is sufficient evidence to conclude that the present material on recruit spawning Atlantic cod shows that (1) fish held on high ration throughout the year will generally spawn earliest and (2) a reduced food supply in the autumn will be more effective in postponing spawning than a similar reduction in the spring. The second point is new, while the first gives support to the findings in Kjesbu (1994).

In summary, 2-yr-old recruit spawning Atlantic cod (TL ≈ 30–50 cm), hatched and reared in the laboratory, held on different feeding regimes for a full year prior to examination, were found to produce 0.25–2.5 million vitellogenic oocytes. The fecundity was positively influenced by growth rate at the time of vitellogenesis (autumn) but insignificantly by growth rate in the foregoing period (spring). Fish with large starting (adolescent) body weights ended as the largest ones. Thus, fecundity was to a great extent affected by the fish's starting weight, shown by multiple regression analysis with adolescent body weight as one of the variables. Large individual variations in number and growth of oocytes suggest strong influence by a genetic component. There was no significant difference in mean relative fecundity among feeding regimes; the noted differences in mean fecundity were positively correlated with mean final fish size. There are indications that fecundity in recruit spawners is less influenced by feeding regime than fecundity in repeat spawners. Favourable feeding conditions, particularly during the autumn, are shown to advance the time of spawning in the year.

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# Shift in the Intestinal Microflora of Atlantic Halibut (*Hippoglossus hippoglossus*) Larvae during First Feeding

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Bergh, Ø., K.E. Naas, and T. Harboe. 1994. Shift in the intestinal microflora of Atlantic halibut (*Hippoglossus hippoglossus*) larvae during first feeding. Can. J. Fish. Aquat. Sci. 51: 1899–1903.

Isolates of aerobic intestinal bacterial flora from unfed Atlantic halibut (*Hippoglossus hippoglossus*) larvae revealed a population dominated by nonfermentative rods of the *Cytophaga/Flexibacter/Flavobacterium* group. Following the onset of feeding, the flora gradually changed towards a fermentative flora dominated by the *Vibrio/Aeromonas* group. This transition occurred faster in a group which was reared in water containing cultivated phytoplankton, in contrast with groups where the larvae were kept in filtered water. No major differences in the bacterial flora of the tank water were found among the groups.

Des isolats de flore intestinale aérobie provenant de larves de flétan atlantique (*Hippoglossus hippoglossus*) non alimentées ont révélé une population dominée par des bâtonnets non fermentatifs appartenant au groupe *Cytophaga/Flexibacter/Flavobacterium*. Après le début de l'alimentation, la flore s'est graduellement modifiée pour devenir une flore fermentative dominée par le groupe *Vibrio/Aeromonas*. Cette transition s'est produite plus rapidement chez un groupe qui a été élevé dans de l'eau renfermant du phytoplancton cultivé, contrairement aux groupes dont les larves ont été gardées dans de l'eau filtrée. On n'a observé aucune différence importante dans la flore bactérienne présente dans l'eau des bassins renfermant les divers groupes.

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An intestinal microflora is established in fish larvae during the yolk sac stage, before active feeding commences (Hansen et al. 1992b; Olafsen and Hansen 1992). Several possible functions have been assigned to the intestinal microflora of fish, including digestion of algal cells (Rimmer and Wiebe 1987), provision of fatty acids (Ringø et al. 1992), amino acids (Fong and Mann 1980), or vitamins (Kashiwada and Teshima 1966), and inhibition of bacterial pathogens (Westerdahl et al. 1991; Olsson et al. 1992). Due to influx of food items, and activation of the gastrointestinal tract, the range of possible roles of the intestinal microflora increases during transition of the larvae to exogenous feeding. Thus, changes in the intestinal flora may constitute an important factor influencing larval survival during this critical period.

The factors controlling the composition, and the functioning, of the intestinal microflora are poorly understood. Environmental factors are believed to influence the composition of the intestinal microflora (Cahill 1990). Algal exudates influence the water-column bacteria (Bird and Kalff 1984; Cole et al. 1988), and some exudates possess inhibitory effects against bacteria (Austin et al. 1992). Effects of algal exudates on the intestinal microflora have been suggested as a major cause of the reported beneficial effects of the so-called green-water technique, involving suspension of phytoplankton in the rearing water of halibut larvae (Skjermo and Vadstein 1993).

The purpose of the present study was to describe the composition of the aerobic intestinal microflora in larvae of the Atlantic halibut (*Hippoglossus hippoglossus*) during first feeding, in relation to the abundance of phytoplankton in the culture medium.

## Materials and Methods

### First-Feeding Experiment

The larvae used for the present study were obtained from an experiment concerning effects of phytoplankton on first feeding of halibut larvae, described in more detail by Naas et al. (1992). The yolk sac larvae were reared in an upwelling incubator with a volume of 15 $m^3$ ("silo") at 7°C for 33 d after hatching, according to Harboe et al. (1994). At this stage, 750 larvae were transferred to each of three outdoor tanks (Ca, Cb, and G, Table 1) in which they were kept for 21 d. These tanks were 1.5 m in diameter and 1 m high. The water flow in the tanks was 1–2 $L \cdot min^{-1}$. The temperature in the outdoor tanks was 9–10.5°C at day 1, decreased rapidly to approximately 6°C at day 3, increased gradually to 12°C at day 9, and was kept in the range of 10–14°C throughout the rest of the experiment.

Two different water qualities were used: tanks Ca and Cb were supplied with "clear water" piped from 55 m depth and filtered through sand (10 μm) and cartridge (5 μm), and tank G was supplied with "green water" from a 280-$m^3$ outdoor tank in which natural phytoplankton production was stimulated through fertilization. The concentration of algae in tank G ranged between one and two orders of magnitude higher than in tanks Ca and Cb throughout the experiment: $7 \times 10^3$ to $90 \times 10^3$ $cells \cdot L^{-1}$ compared with $0.4 \times 10^3$ to $13 \times 10^3$ $cells \cdot L^{-1}$, respectively. Flagellates smaller than 5 μm in diameter dominated both populations (Naas et al. 1992).

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TABLE 1. Water source of the incubators, sampling times (days after transfer to start-feeding units), numbering of the groups of isolated strains, and mean larval myotomal height (μm) with standard deviation.

| Incubator | Water source of incubator | Sampling time | Numbering of bacterial strains: from larvae | Numbering of bacterial strains: from incubator water | Mean myotomal height (μm) (SD) |
|---|---|---|---|---|---|
| Silo | Seawater (55 m) | Day 0 | L-0 | W-0 | 878 (37) |
| Tank Ca | Seawater (55 m) | Day 7 | L-Ca1 | W-Ca1 (lost) | 780 (31) |
| | | Day 14 | L-Ca2 | W-Ca2 | 1017 (163) |
| | | Day 21 | L-Ca3 | W-Ca3 | 1026 (290) |
| Tank Cb | Seawater (55 m) | Day 7 | L-Cb1 | W-Cb1 | 750 (31) |
| | | Day 14 | L-Cb2 | W-Cb2 | 873 (94) |
| Tank G | Phytoplankton basin | Day 7 | L-G1 | W-G1 | 766 (70) |
| | | Day 14 | L-G2 | W-G2 | 1536 (400) |
| | | Day 21 | L-G3 | W-G3 | 1800 (437) |

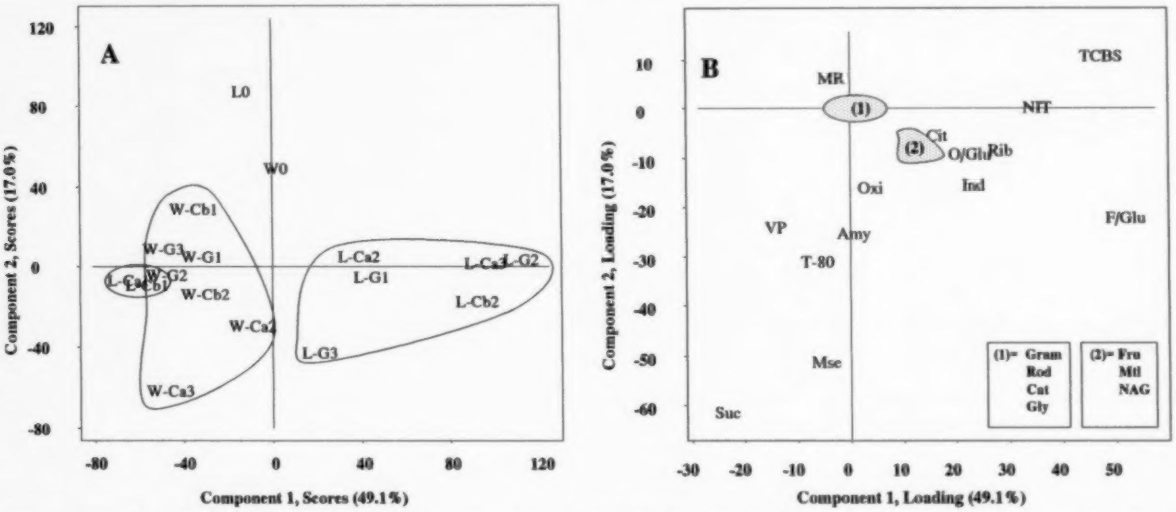

FIG. 1. Principal components analysis of the frequencies of positive biochemical and morphological tests. (A) Score plot of the characteristics of the microflora in the different larvae (L) and water (W) groups. (B) Corresponding plot of the variables (test types) explaining the variance illustrated in Fig. 1A. The tests Gram, Rod, Cat, and Gly had almost identical coordinates and are represented by a marked area positioned close to origo, marked with (1). Similarly, the tests Fru, Mtl, and NAG are represented by the area marked with (2). For explanations of the different groups of bacteria from larvae and water and their abbreviations, see Table 1. The abbreviations of the different tests are given in Tables 2 and 3.

In tanks Ca and G, the larvae were fed unenriched *Artemia* instar II (Artemia Systems, Gent, Belgium) whereas larvae in tank Cb were fed *Artemia* instar II which in advance had been stored for 6 h in green water.

### Isolation of Bacteria

Isolation of intestinal bacteria was carried out by a method modified from Muroga et al. (1987). Five to 10 live larvae were washed three times in autoclaved seawater adjusted to a salinity of 25 ppt (SSW). The larvae were surface treated with an iodophor disinfectant (0.05% Buffodine$^{TM}$, Evans Vanodine, Preston, U.K.) for 3 min, washed again three times in SSW, and homogenized together in 5 mL of SSW. A dilution series was made in SSW and plated out on Petri dishes with Difco, 2216 Marine Broth with 1.5% agar (MBA) (Difco, Detroit, Mich.). After incubation at 10°C for 14 d, 48–64 strains were randomly picked for phenotypical characterization. The sampling times and the origin and numbering of each group of isolated are listed in Table 1.

Isolation of bacteria from water in the rearing tanks was carried out by making a dilution series in SSW followed by plating out on MBA Petri dishes. The sampling times (Table 1), incubation, and selecton of strains were identical to the isolations of intestinal bacteria. At the end of the experiment (day 21), no larvae were alive in tank Cb; thus, sampling in this tank was restricted to days 7 and 14 (Table 1).

### Phenotypical Characterization of the Isolates

Gram staining was carried out with the Bacto Gram stain set (Difco, Detroit, Mich.) and the oxidase test with the API 7046 kit (Bio Merieux, France). The catalase test was carried out with 3% $H_2O_2$. The strains were categorized morphologically as rods or cocci.

The following tests were adapted to an incubation

TABLE 2. Percentage of positive tests among the isolates from the groups isolated from larvae. The abbreviations of the tests are used in Fig. 1B.

| Group | L-0 | L-Ca1 | L-Ca2 | L-Ca3 | L-Cb1 | L-Cb2 | L-G1 | L-G2 | L-G3 |
|---|---|---|---|---|---|---|---|---|---|
| Gram | 2.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rod | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Oxidase (Oxi) | 86.5 | 95.8 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Catalase (Cat) | 100 | 95.8 | 95.7 | 100 | 97.7 | 100 | 100 | 100 | 95.3 |
| TCBS | 56.5 | 4.2 | 89.1 | 77.8 | 14.0 | 95.7 | 72.9 | 89.1 | 7.8 |
| Oxidative glucose (O/Glu) | 0 | 4.2 | 4.3 | 36.5 | 0 | 51.1 | 47.9 | 32.7 | 28.1 |
| Fermentative glucose (F/Glu) | 0 | 4.2 | 37.0 | 85.7 | 9.3 | 85.1 | 68.8 | 98.2 | 65.6 |
| Acid produced aerobically from: | | | | | | | | | |
| *N*-acetylglucosamine (NAG) | 0 | 0 | 15.2 | 22.2 | 4.7 | 38.3 | 20.8 | 25.5 | 9.4 |
| fructose (Fru) | 0 | 0 | 13.0 | 23.8 | 0 | 23.3 | 20.8 | 20.0 | 10.9 |
| ribose (Rib) | 13.5 | 4.2 | 5.0 | 27.0 | 4.7 | 48.9 | 29.2 | 85.5 | 15.6 |
| sucrose (Suc) | 0 | 95.8 | 54.3 | 22.2 | 74.4 | 53.2 | 43.8 | 36.4 | 59.4 |
| mannitol (Mtl) | 0 | 4.2 | 21.7 | 11.1 | 0 | 29.8 | 25.0 | 25.5 | 4.7 |
| mannose (Mse) | 2.7 | 4.2 | 21.7 | 33.3 | 39.5 | 21.3 | 47.9 | 20.0 | 54.7 |
| glycerol (Gly) | 0 | 0 | 0 | 9.5 | 0 | 2.1 | 4.2 | 14.5 | 3.1 |
| Indole (Ind) | 0 | 0 | 41.3 | 44.4 | 0 | 40.4 | 22.9 | 21.8 | 50.0 |
| Methyl Red (MR) | 100 | 95.8 | 100 | 88.9 | 100 | 100 | 100 | 100 | 73.4 |
| Voges–Proskauer (VP) | 2.7 | 20.8 | 15.2 | 11.1 | 18.6 | 44.7 | 2.1 | 7.3 | 45.3 |
| Citrate (Cit) | 0 | 0 | 0 | 25.4 | 0 | 25.5 | 0 | 38.2 | 21.9 |
| Nitrate reductase (NIT) | 24.3 | 12.5 | 67.4 | 90.5 | 7.0 | 51.1 | 10.4 | 65.5 | 70.3 |
| Degradation of Tween 80 (T-80) | 62.2 | 95.8 | 93.5 | 95.2 | 95.3 | 95.7 | 79.2 | 63.6 | 95.3 |
| Amylase (Amy) | 54.1 | 100 | 95.7 | 85.7 | 90.7 | 70.2 | 85.9 | 100 | 92.2 |

temperature of 10°C and otherwise carried out according to the method described by Hansen and Sørheim (1991): oxidative/fermentative metabolism of glucose, aerobic acid production from fructose, sucrose, mannitol, mannose, glycerol, ribose, and *N*-acetylglucosamine, gelatinase, nitrate reductase, Voges–Proskauer, methyl red, production of indole from tryptophane, and degradation of Tween 80.

The citrate test was carried out with 2% NaCl added to Simmons' citrate medium. The strains were checked for growth on Tryptone Citrate Bile Salt agar (TCBS, Oxoid, Basingstoke, U.K.). The oxidative carbohydrate tests were incubated for 10 d, the fermentative glucose test for 14 d and the indole, Voges–Proskauer, methyl red, nitrate reductase, and TCBS tests for 14 d whereas the citrate tests were incubated for 4 wk. All tests were incubated at 10°C.

Principal component analysis was run on the percentages of positive tests (21 variables) for each of the groups (17 objects) isolated from incubator water and larval intestines. The computer program Sirius (Kvalheim and Karstang 1987) was used for this purpose.

## Results

No significant differences in mean myotomal height could be detected on day 7 among larvae in the three tanks. From day 7 onwards, however, the larvae in tank G were larger than in the clear-water tanks (Table 1). Larvae in tank G also had the highest survival at the end of the experiment (day 21), 9.75% compared with 4.17% in tank Ca and 0% in tank Cb.

All the isolated bacteria from both water and intestines were rod shaped, all but one strain were Gram negative, and most were oxidase and catalase positive.

The principal components analysis (Fig. 1A) demonstrates that all groups of bacteria isolated from tank water were relatively similar, and groups L-Ca1 and L-Cb1 clustered into the same area of the plot. In contrast with this, all groups of intestinal bacteria isolated from larvae in tank G and the remaining groups isolated from larvae in tanks Ca and Cb had relatively higher frequencies of strains positive for fermentative glucose, nitrate reductase, and TCBS (Fig. 1B). All these groups were different from the groups of bacteria isolated from unfed larvae (group L-0), indicating that a time-dependent succession had occurred.

The flora from yolk sac larvae was dominated by strains that possessed the characteristics of the *Cytophaga/Flexibacter/Flavobacterium* group. These bacteria were long rods, some of which possessed gliding motility. They were not fermentative and did not produce acid aerobically from any of the carbohydrates tested, with a few exceptions regarding utilization of ribose. No strains possessing the characteristic pale yellow pigmentation of *Flexibacter ovolyticus* (Hansen et al. 1992a) were present.

There were several differences among the populations isolated from larvae from different tanks at different sampling times (Table 2). Fermentative strains were not found in group L-0, and they constituted less than 10% of the groups isolated from larvae in tanks Ca and Cb on day 7 after first feeding (L-Ca1 and L-Cb1). In later samplings from tanks Ca and Cb, the frequency of fermentative strains increased. In all samplings from tank G, fermentative strains constituted more than 60% of the isolates.

A similar pattern was found regarding the ability to produce acid aerobically from different carbohydrates. This ability was rare in groups L-0, L-Ca1, and L-Cb1, with the exception of sucrose as substrate in groups L-Ca1 and L-Cb1 and mannose in group L-Cb1. All samplings from tank G and the later samplings in tanks Ca and Cb showed a large number of strains able to produce acid from most

TABLE 3. Percentage of positive tests among the isolates from the groups isolated from incubator water. The abbreviations of the tests are used in Fig. 1B.

| Group | W-0 | W-Ca2 | W-Ca3 | W-Cb1 | W-Cb2 | W-G1 | W-G2 | W-G3 |
|---|---|---|---|---|---|---|---|---|
| Gram | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rod | 100 | 100 | 98.3 | 100 | 100 | 100 | 100 | 100 |
| Oxidase (Oxi) | 95.8 | 100 | 100 | 52.1 | 100 | 100 | 100 | 95.3 |
| Catalase (Cat) | 91.7 | 90.6 | 98.3 | 93.8 | 96.2 | 95.1 | 100 | 86.0 |
| TCBS | 41.7 | 39.6 | 33.3 | 16.7 | 50.9 | 17.1 | 18.8 | 9.3 |
| Oxidative glucose (O/Glu) | 25.0 | 9.4 | 11.7 | 6.3 | 0 | 17.1 | 10.4 | 7.0 |
| Fermentative glucose (F/Glu) | 22.9 | 35.8 | 11.7 | 25.0 | 11.3 | 19.5 | 12.5 | 16.3 |
| Acid produced aerobically from: | | | | | | | | |
| *N*-acetylglucosamine (NAG) | 2.1 | 1.9 | 11.7 | 2.1 | 0 | 19.5 | 8.3 | 2.3 |
| fructose (Fru) | 6.3 | 3.8 | 6.7 | 0 | 1.9 | 2.4 | 9.2 | 0 |
| ribose (Rib) | 0 | 18.9 | 11.7 | 4.2 | 11.3 | 4.9 | 10.4 | 2.3 |
| sucrose (Suc) | 22.9 | 77.4 | 93.3 | 37.5 | 86.8 | 43.9 | 70.8 | 55.8 |
| mannitol (Mtl) | 2.1 | 3.8 | 10.0 | 0 | 1.9 | 7.3 | 0 | 2.3 |
| mannose (Mse) | 10.4 | 67.9 | 88.3 | 12.5 | 18.9 | 48.8 | 27.1 | 27.9 |
| glycerol (Gly) | 2.1 | 0 | 0 | 0 | 0 | 2.4 | 4.2 | 2.3 |
| Indole (Ind) | 0 | 3.8 | 1.7 | 8.3 | 15.1 | 2.1 | 0 | 0 |
| Methyl Red (MR) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Voges–Proskauer (VP) | 33.3 | 3.8 | 71.7 | 50.0 | 49.1 | 4.9 | 27.1 | 55.8 |
| Citrate (Cit) | 6.3 | 0 | 1.7 | 0 | 0 | 0 | 0 | 0 |
| Nitrate reductase (NIT) | 58.3 | 39.6 | 13.3 | 18.8 | 24.5 | 9.8 | 2.1 | 20.9 |
| Tween 80 (T-80) | 50.0 | 96.2 | 100 | 95.8 | 96.2 | 82.9 | 87.5 | 81.4 |
| Amylase (Amy) | 81.3 | 98.1 | 90.0 | 64.6 | 94.3 | 95.1 | 95.8 | 62.8 |

of the carbohydrates tested. Citrate utilizers were isolated only from the late samplings of the experiment, in groups L-Ca3, L-Cb2, L-G2, and L-G3. Strains possessing nitrate reductase were present in all samplings, but the frequency increased towards the end of the experiment.

The flora isolated from tank water (Table 3) differed in some respects from the intestinal isolates. The percentage of fermentative strains was relatively lower compared with the later samples of intestinal bacteria. However, such strains were present in the water in relatively constant amounts throughout the experiment. Similarly, a low number of strains possessing nitrate reductase were found throughout the experiment. An exception was the incubation water of the incubator for yolk sac larvae (group W-0), which possessed 58.3% strains with nitrate reductase. A low number of citrate utilizers were present among the isolates from the water. Strains producing acid from a range of carbohydrates were present throughout the experiment.

In contrast with the intestinal flora, no time-dependent succession patterns could be identified in the tank water flora. No major differences among the water floras of tanks Ca, Cb, and G were evident. One exception was found in tank Cb, as group W-Cb1 had a high frequency of oxidase-negative strains. This was not found anywhere else during the experiment.

## Discussion

Our results demonstrate a succession in the composition of the intestinal microflora that occurred in all groups during the experimental period. The flora was dominated at the outset by nonfermentative bacteria, which were gradually replaced by fermentative strains, capable of producing acid from a wide range of carbohydrates. The shift occurred faster in larvae from the tank supplied with green water compared with larvae kept in clear-water tanks.

These data indicate that the onset of feeding is the time of transition of the intestinal flora of halibut between two kinds of populations, similar to the shift demonstrated in the intestinal flora of masu salmon (*Oncorhynchus masou*) and chum salmon (*Oncorhynchus keta*) (Yoshimizu et al. 1980). When the digestive tract was activated, the intestinal flora of these species changed from a *Pseudomonas*-dominated flora towards a flora dominated by *Aeromonas* strains.

Taxonomically, our results seem consistent with earlier findings from marine fish. Bacteria belonging to the *Cytophaga/Flexibacter/Flavobacterium* group have been shown to be associated with marine fish eggs (Hansen et al. 1992a) and larvae prior to first feeding (Hansen et al. 1992b). In adult marine fish of many species, *Vibrio* spp., and to some extent *Pseudomonas* spp., seem commonly to dominate the intestinal flora (Sakata 1990). *Vibrio* spp. may also dominate the intestinal microflora of fed larvae of other marine fish species such as red seabream (*Pagrus major*) and black seabream (*Acanthopagrus schlegeli*) (Muroga et al. 1987) and turbot (*Scophthalmus maximus*) (Nicolas et al. 1989).

MacFarlane et al. (1986) noted differences in the composition of the intestinal floras of marine and estuarine striped bass (*Morone saxatilis*), perhaps reflecting a higher organic content of the estuarine water as compared with marine water. In our experiment, the water flora differed in many respects from the intestinal flora; thus, to a certain extent, a specific intestinal flora was present at all stages of the experiment. However, despite the differences in phytoplankton concentration, the differences among the water flora were low compared with those among the populations isolated from the larvae.

The changes found by Skjermo and Vadstein (1993) in the intestinal flora of halibut larvae caused by addition of algae were mainly related to antibiotic susceptibility. Following their hypothesis, the higher amounts of algae in tank G compared with tanks Ca and Cb might have caused alterations in the intestinal microflora of the larvae. However, the relative similarity among the populations isolated from the water in the three tanks indicates that other factors may be quantitatively more important in explaining the differences regarding intestinal flora.

Addition of algae to the culture medium also influences the light regime, and thereby probably the contrast and the visibility of the prey items, increasing the feeding efficiency of the larvae (Naas et al. 1992). Increased feeding efficiency would imply an increased influx of bacteria associated with the food items. The composition of the intestinal microflora also seems to vary with the complexity of the fish digestive system (Cahill 1990). Thus, the observed size differences at sampling times 14 and 21 d between the larvae in tank G compared with tanks Ca and Cb may have contributed to the differences in intestinal microflora. The different speed at which the transition occurred in tank G compared with tanks Ca and Cb would also indicate that feeding efficiency may be a quantitatively important factor contributing to the observed differences in intestinal flora.

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# CORRECTIONS TO RECENT PUBLICATIONS
# CORRECTIONS SUR RECENTES PUBLICATIONS

VOLUME 45, No. 6 — article by R.I.C.C. Francis: *Are growth parameters estimated from tagging and length–age data comparable?*

Page 938: the equation immediately following equation (3) should read as follows:

$$\lambda = \begin{cases} (2c)/[\log(l_\infty + c) - \log(l_\infty - c)] \text{ if } L < l_\infty - c \\ (l_\infty + c - L)/[\log(l_\infty + c) - \log(L)] \text{ if } L > l_\infty - c. \end{cases}$$

With this correction, the expected annual growth for fish of length 69.9 cm is 9.5 cm, not 11 cm (as given in the penultimate paragraph of this section). The author is indebted to You-gan Wang (CSIRO, Department of Fisheries, Australia) for pointing out this error.

VOLUME 50, No. 11 — article by M.T. Arts, R.D. Robarts, and M.S. Evans: *Energy reserve lipids of zooplanktonic crustaceans from an oligotrophic saline lake in relation to food resources and temperature.*

Page 2418: Due to printer error, the last row in Table 3 was omitted. Table 3 should read as follows:

TABLE 3. Physical and biological variables in relation to total and energy reserve lipid content identified by forward stepwise regression (*F* value to enter = 4). EP = the proportion of the primary productivity estimated to be due to edible algal biomass in the epilimnion.

| Model ($r^2$) | Dependent variable | Coefficient | SE | *t*-value | Significance level |
|---|---|---|---|---|---|
| Total lipid (0.92) | Constant | 22.11 | 3.47 | 6.37 | <0.001 |
| | Epilimnetic temperature | −1.21 | 0.16 | −7.34 | <0.001 |
| | Epilimnetic edible biomass | 0.04 | 0.01 | 6.30 | <0.001 |
| | Hypolimnetic temperature | 2.01 | 0.31 | 6.40 | <0.001 |
| Triacylglycerol (0.88) | Constant | 2.74 | 0.62 | 4.46 | <0.001 |
| | Epilimnetic temperature | −0.16 | 0.03 | −5.16 | <0.001 |
| | Epilimnetic edible biomass | 0.04 | 0.001 | 4.93 | <0.001 |
| | EP | 0.29 | 0.13 | 2.20 | 0.043 |

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# Le flétan de l'Atlantique (*Hippoglossus hippoglossus*) et le flétan du Pacifique (*H. stenolepis*) et les pêcheries de ces espèces en Amérique du Nord

Dans le Bulletin canadien des sciences halieutiques et aquatiques nº 227F

Le flétan de l'Atlantique (*Hippoglossus hippoglossus*) et le flétan du Pacifique (*H. stenolepis*) qui vivent au large des côtes de l'Atlantique et du Pacifique de l'Amérique du Nord ont de nombreuses caractéristiques biologiques en commun. La séparation traditionnelle du flétan en espèces distinctes n'est pas uniformément acceptée. La reproduction et les premiers stades de vie sont presque identiques, bien que la dérive sur une grande distance des oeufs et des larves de flétan du Pacifique et la contre-migration des juvéniles n'aient pas été mises en évidence dans le cas du flétan de l'Atlantique. Les flétans adultes migrant depuis les aires de frai d'hiver situées le long de la partie supérieure du talus continental pour se rendre dans les aires d'alimentation d'été situées sur le plateau continental. Les deux espèces de flétan montrent les caractéristiques semblables du point de vue de l'âge et de la croissance. Elles présentent une sélectivité trophique différent du fait que,comparativement au flétan du Pacifique, le flétan de l'Atlantique de petite taille consomme une proportion plus élevée d'invertébrés et le flétan de l'Atlantique de grande taille consomme une proportion plus élevée de poissons. La gestion du flétan dans l'Atlantique et dans le Pacifique est totalement différente. 96 pages **(Disponible en anglais)**

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# Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) and their North American fisheries

**In the Canadian Bulletin of Fisheries and Aquatic Sciences No. 227E**

Atlantic halibut (*Hippoglossus hippoglossus*) and Pacific halibut (*H. stenolepis*) off the Atlantic and Pacific coasts of North America share many biological characteristics. The conventional separation of the halibut into separate species is not uniformly accepted. Reproduction and early life history are nearly identical, although the extensive drift of Pacific halibut eggs and larvae and countermigration as juveniles has not been demonstrated for the Atlantic halibut. Adult halibut undergo migration from winter spawning grounds along the upper continental slope to summer feeding grounds on the continental shelf. The two halibuts show the same pattern of age and growth. They exhibit different trophic selectivity, as small Atlantic halibut consume a higher proportion of invertebrates as small fish and large Atlantic halibut consume a higher proportion of fish, compared to Pacific halibut. Halibut management in the Atlantic and Pacific varies dramatically. **(Available in French)**

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# Canadian Journal of Fisheries and Aquatic Sciences

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